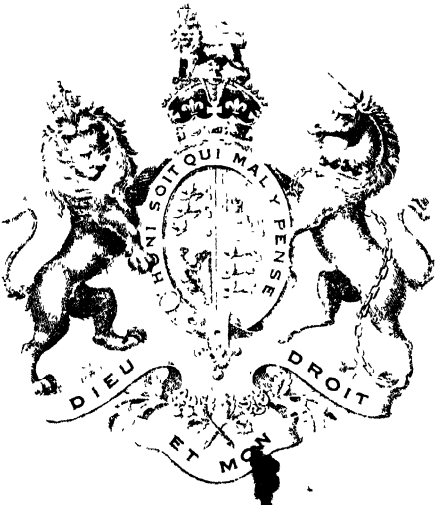

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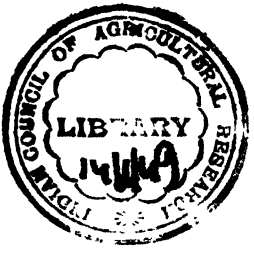

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STORAGE OF COTTONSEED AND PEANUTS UNDER CONDITIONS WHICH MINIMIZE CHANGES IN CHEMICAL COMPOSITION [1]

By MACK F. STANSBURY, *assistant chemist*, and JOHN D. GUTHRIE, *senior chemist*, *Southern Regional Research Laboratory, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture* [2]

INTRODUCTION

In the investigation of the chemical composition of cottonseed and peanuts, a method of storage which holds changes in chemical composition to a minimum is needed whenever samples are collected in considerable numbers and all cannot be worked on at the same time. Such a method of storage would also make it possible to keep at hand a collection of typical samples needed for investigative work extending over a considerable period of time. The chief object of the present investigation was to find a satisfactory method for storing cottonseed samples for at least a year without appreciable change in chemical composition. From a consideration of reports in the literature (*4*),[3] it would seem that the storage of peanuts does not present as difficult a problem as cottonseed storage. Nevertheless, a few samples of peanuts were included in the storage experiments.

Most seeds retain their viability best when kept dry and at low temperatures (*3*, *5*). Simpson (*10*, *11*) found this to be true for cottonseed. He also showed that little or no increase in free fatty acids occurred when cottonseed with a moisture content of less than 9 percent was stored for 2 years at 21° C. When cottonseed samples were stored at 1°, little or no increase in the free fatty acid content took place even at a moisture content of 14 percent. With the literature on seed viability and Simpson's experiments on cottonseed as a guide, it seemed likely that a satisfactory method for the storage of cottonseed would be to dry the samples to a moisture content of 8 percent or less and store at a temperature of 1° or below. However, the possibility that storage at a moisture content of 8 percent or less at room temperature (25°–28°) might prevent any appreciable change in chemical composition made it advisable to include this method of storage as well.

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[2] The authors wish to thank H. W. Barre and D. M. Simpson of the Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, for supplying the cottonseed, and B. B. Higgins, of the Georgia Agricultural Experiment Station, for supplying the peanuts. The authors are also indebted to Walter A. Pons, Jr., Vidabelle Orr, Richard H. Robinson, Alva Faust, and Dorothy H. Petty for aid in making some of the chemical analyses.

[3] Italic numbers in parentheses refer to Literature Cited, p. 61.

SOURCES OF SAMPLES

The cottonseed samples were obtained from the field deterioration studies of the Bureau of Plant Industry, Soils, and Agricultural Engineering. These samples included successive pickings from six varieties grown at six locations in the Cotton Belt in 1941 and seven varieties from a similar experiment in 1942. A few samples from the regional storage tests of the Bureau of Plant Industry, Soils, and Agricultural Engineering were used in some of the experiments.

Four samples of peanuts were obtained from the Georgia Agricultural Experiment Station.

METHOD OF STORAGE

Most of the cottonseed samples had a moisture content of between 7 and 8 percent at the beginning of the experiment. A few samples which contained more than 8.3 percent moisture were dried before storage by placing them in a closed 30° C. forced-draft circulatory oven containing a tray of anhydrous calcium chloride until the moisture content of the seeds reached about 7 percent.[4] The samples were mixed thoroughly, divided into four equal portions, and each portion was placed in a sealed container. One-gallon tin cans were used for the larger samples. These were sealed with paraffin. Mason jars were used for the smaller samples. One part of the sample was analyzed, and the other three parts were stored at room temperature, in commercial cold storage at 1°, and at −18° C.

The samples of unshelled peanuts were stored in closed, but unsealed, metal cans at room temperature, at 1°, and at −18° C.

METHODS OF ANALYSIS

Moisture, total nitrogen, oil, iodine number of the oil, and free fatty acid content of the oil were determined by the methods of the American Oil Chemists' Society (*1*). The method of Greenbank and Holm (*6*) was used for the determination of the peroxide number of the oil. Other methods are described in the following paragraphs.

FREE FATTY ACIDS IN THE OIL

Some of the cottonseed samples were too small for free fatty acid determinations by the method of the American Oil Chemists' Society. For these samples the following method, developed by Carroll L. Hoffpauir of this laboratory, was used:

Duplicate 2-gm. samples of kernels were obtained by removing the hulls. The kernels were cut fine or crimped and shaken with 100 ml. of Skellysolve F for 2 hours on a mechanical shaker. The extracted oil was obtained by evaporation on the steam bath of 50-ml. portions of the extract filtered into tared 100-ml. oil-extraction flasks. After the weight of the oil was determined, it was dissolved in 10 ml. of Skellysolve F and 25 ml. of redistilled 95-percent ethyl alcohol. About 5 drops of 0.05-percent *m*-cresol purple indicator were added,

[4] The possibility that drying in this way might affect the seeds adversely was checked by the determination of catalase activity before and after drying No appreciable change in catalase activity was found.

and the solution was titrated with 0.01 N alcoholic NaOH until a purple color appeared. A stream of CO_2-free air was bubbled through the solution during the titration. Blanks were run on the reagents and corrections made.

REDUCING SUGARS

Approximately 100 gm. of cottonseed were dried for 30 minutes at 100° to 105° C., cooled, and dehulled in a Bauer mill. The kernels were separated by screening and ground to a fine meal through the Bauer mill. Approximately 40 gm. of the ground kernels were weighed into extraction thimbles and extracted in a Soxhlet apparatus with Skellysolve F for 8 hours to remove most of the oil. The material was then extracted for 20 hours with 70-percent ethyl alcohol. The alcohol was removed from the extracts by heating on the steam bath with frequent addition of water, and the extracts were transferred to 500-ml. volumetric flasks, leaded, made to volume, and deleaded in the customary manner. Reducing sugars were determined on 50-ml. aliquots by precipitating cuprous oxide according to the Munson-Walker general method (*2, p. 500*), followed by use of the volumetric permanganate method of the Association of Official Agricultural Chemists (*2, p. 501 502*). Reducing sugars were calculated as glucose.

TOTAL SUGARS

Aliquots of the deleaded extracts prepared for the reducing-sugar determinations were used for the determination of total sugars, including raffinose. Aliquots of 100 ml. were placed in 250-ml. flasks and hydrolyzed by heating under reflux condensers for $2\frac{1}{2}$ hours on the steam bath with 10 ml of HCl (sp gr. 1.125). This long hydrolysis was necessary to insure complete hydrolysis of raffinose. The solutions were cooled, neutralized with NaOH, and made to 250 ml. in volumetric flasks Sugars were determined on 50-ml. aliquots in the same manner as for reducing sugars, and calculated as invert sugar. In some of the total sugar determinations, the 70-percent alcoholic extract of oil-free kernels prepared for protein solubility determinations was used.

RAFFINOSE

Aliquots of the deleaded extracts prepared for the reducing sugar determinations were used for the determination of raffinose in the presence of sucrose by the double enzymatic hydrolysis method of the Association of Official Agricultural Chemists (*2, p. 495*). Little, if any, sucrose was found in the samples of cottonseed kernels analyzed. The invertase was prepared from baker's yeast and the invertase-melibiase from brewer's yeast. Both solutions were concentrated by ultrafiltration (*2, p. 492*).

CATALASE

Approximately 8 gm. of cottonseed were obtained by mixing and reducing the entire sample. The seeds were split by hand, the kernels were removed, chopped fine, mixed thoroughly, and 1 gm. was transferred to a Waring blendor. The kernels were ground with 75 to 100 ml. of water, the sides of the blendor jar being washed down periodically with a little water. When a uniform suspension was obtained,

it was transferred to a volumetric flask and made to 200 ml. Catalase activity was determined immediately on 5-ml. aliquots, by using a modification by Davis of Appleman's apparatus described in Miller's Plant Physiology (7). Determinations were made at 25° C. with 5 ml. of extract, 5 ml. of pH 7.1 phosphate buffer, and 5 ml. of 3-percent H_2O_2 neutralized with $CaCO_3$. Volumes of oxygen were read at 1-, 2-, and 3-minute intervals, but only the 2-minute values will be reported here.

PROTEIN SOLUBILITY

The Skellysolve-F-extracted residues from the free fatty acid determinations were air-dried, ground to pass a 16-mesh screen, and extracted with diethyl ether for 36 hours in a Soxhlet extractor. The oil-free residue was ground to pass a 60-mesh screen, and hull fragments were removed by screening. The material was allowed to air-equilibrate and the relative protein solubility was determined by the method of Olcott and Fontaine (8). Results were calculated as percent of the total nitrogen peptized by 0.5 N NaCl solution.

EXPERIMENTAL RESULTS

Approximately 100 samples of cottonseed from the 1941 field deterioration studies of the Bureau of Plant Industry, Soils, and Agricultural Engineering were stored. The free fatty acid content of the oil was determined on all the samples at the time of storage and after storage. After the elimination of data on certain samples because of questionable accuracy of the original free fatty acid values, as indicated by their failure to correlate with germination values supplied with the samples, and the elimination of other samples too small to be included in all three storage conditions, the samples were grouped according to the original free fatty acid values and averages were calculated for each storage condition. The summary of these results is given in table 1. A small increase in the free fatty acid content occurred in the samples stored at room temperature (25°–28°), but no increase was observed in the samples stored at 1° and at −18° C. The apparent decrease in free fatty acids observed in some of the samples stored at −18° might not have been a real decrease, but possibly one arising from the difficulties inherent in the sampling of cottonseed. This conclusion received support from the data obtained in subsequent experiments, especially those reported in table 5.

TABLE 1.—*Free fatty acid content of the oil after storage of cottonseed in sealed containers at various temperatures*

Method	Range of free fatty acid content of oil	Samples	Average free fatty acid content of oil			
			Start of test	18 months 25°–28° C.	18 months 1° C	18 months −18° C
	Percent	*Number*	*Percent*	*Percent*	*Percent*	*Percent*
A. O. C. S	0 1 to 0 3	25	0 2	0.3	0.2	0 2
Do	3 to 2.2	2	5	.8	5	5
Do	2 2 to 30.0	3	19 3	22 8	18 5	16 7
Micro	1 to .3	15	2	3	2	.1
Do	3 to 2.2	12	1 4	2 2	1 3	1 0
Do	2 2 to 12 0	6	7.2	7 9	5 9	

Five of the larger samples from the 1941 field deterioration studies were selected to cover the range of free fatty acid content of the entire group of samples. The results of analyses made for free fatty acid content of the oil, iodine number of the oil, peroxide number of the oil, total nitrogen content of the kernels, and catalase activity of the kernels are given in tables 2 and 3. The data show a small increase in the free fatty acid content of the oil in the samples stored for 18 months at room temperature, but no increase in the samples stored at 1° or −18° C. A small decrease in the iodine number of the oil may have occurred in the samples stored at room temperature, but otherwise no appreciable change in iodine number, peroxide number, total nitrogen content, or catalase activity took place under any of the storage conditions. It is of interest to note that the catalase activity of high free fatty acid cottonseed was considerably lower than that of low free fatty acid samples.

Six samples of low and high free fatty acid content from the 1941 crop were stored at room temperature for 24 months to ascertain whether or not any change in sugars would take place. The results are shown in table 4. A slight decrease in reducing sugars was observed for all samples. Although the data seem to show a small average increase in raffinose and a decrease in total sugars upon storage at room temperature, it is probable that such changes are due to errors inherent in sampling cottonseed and hydrolyzing sugar extracts and do not represent real changes. It is worth noting that the raffinose content of the kernels of low free fatty acid seeds was approximately 8 percent, as compared with 3 percent for high free fatty acid seeds.

Germination determinations on 10 selected samples stored for 18 months at room temperature, at 1°, and at −18° C. showed no appreciable change from the values found before the samples were shipped to this laboratory.

With the results on the 1941 storage experiments to serve as a guide, 20 samples were selected from the 1942 crop of the field deterioration studies mentioned above. These included seed samples which were high and low in free fatty acids and included 7 varieties of cottonseed. The 20 samples, with moisture content between 7.4 and 8.3 percent, were analyzed for total nitrogen and total oil content of the whole seed, and free fatty acid content of the oil. After storage for 14 months the samples were analyzed again. The results are given in table 5. There was no appreciable change in total nitrogen or oil content during storage, but a significant increase in the free fatty acid content of the oil occurred in the samples stored at room temperature. No significant change in free fatty acid content of the oil was observed in the samples stored at 1° or −18° C. for 14 months.

Seven samples of cottonseed selected for high and low free fatty acid content were analyzed for total sugars and relative protein solubility of oil-free kernels, stored for 14 months at the various temperatures, and analyzed again. The results are given in table 6. Apparently, a small increase in total sugars and in relative protein solubility took place during storage at all temperatures. It is likely, however, that this was due to some systematic error of sampling and analysis and that a significant increase did not occur. Especially is

TABLE 2.—*Analyses of the oil after storage of cottonseed at various temperatures*

Analysis No.	Variety	Place grown	Date of picking	Free fatty acid content of oil				Iodine No. of oil (Wijs)				Peroxide No. of oil			
				At start of test	18 months, 25°–28° C.	18 months, 1° C.	18 months, −18° C.	At start of test	18 months, 25°–28° C.	18 months, 1° C.	18 months, −18° C.	At start of test	18 months, 25°–28° C.	18 months, 1° C.	18 months, −18° C.
				Percent	*Percent*	*Percent*	*Percent*								
A867	Farm Relief (strain 3)	Knoxville, Tenn.	Sept. 30, 1941	0.1	0.2	0.2	0.2	107	105	106	106	4	2	1	2
A874	Stoneville 37	Tifton, Ga.	Aug. 21, 1941	9	1.3	9	8	107	104	106	106	2	2	1	3
A924	do	Stoneville, Miss.	Sept. 26, 1941	2	2	2	2	111	109	110	110	2	2	2	4
AB202	Rowden 42A	Baton Rouge, La.	Sept. 10, 1941	10.9	13.9	11.0	10.1	106	104	106	106	1	1	1	2
AB203	Coker 100 (strain 3).	do	do	35.0	41.3	36.3	33.0	107	106	107	108	1	0	0	1

TABLE 3.— *Total nitrogen content and catalase activity of the kernels after storage of cottonseed at various temperatures*

Analysis No.	Total nitrogen (dry basis)				Catalase (ml O_2 in 2 min.)			
	At start of test	18 months, 25°-28° C.	18 months, 1° C.	18 months, −18° C.	At start of test	18 months, 25°-28° C.	18 months, 1° C.	18 months, −18° C.
	Percent	*Percent*	*Percent*	*Percent*				
A867	6.49	6.58	6.65	6.68	12.9	13.4	13.3	12.1
A874	5.98	6.16	6.14	6.19	13.1	13.8	13.5	11.4
A924	5.02	4.98	5.12	5.14	10.5	11.0	11.0	9.9
AB202	6.35	6.49	6.46	6.45	4.1	5.1	5.7	5.3
AB203	6.83	6.87	6.80	6.80	3.4	4.0	3.9	3.0

this true of total sugars determinations, where acid hydrolysis of the sugar extracts probably gives variable results. Additional data for the protein solubility on 5 samples stored 14 months at room temperature are given in table 7. An increase in relative protein solubility was observed in only 1 of these samples. Very little change was found in the protein solubility of the extracted meal after it was stored for 14 months. This is in agreement with the results of Olcott and Fontaine (*9*). The pH values on the NaCl extracts of the meals were also observed to be relatively unchanged.

The results of an experiment in which four samples of unshelled peanuts were stored in closed, but unsealed, metal cans at room temperature, at 1°, and at −18° C., for 30 months, are shown in tables 8 and 9. A slight increase in the free fatty acid content was observed in room-temperature storage, but no increase occurred at 1° or at −18°. The increase in free fatty acids was very large for one of the samples that molded at room temperature. This sample had been analyzed at the end of 6 months' storage at room temperature and was then in good condition. The total nitrogen and oil content of the kernels and the iodine number of the oil remained relatively constant under all the storage conditions for all the samples except the one that molded at room temperature. Slight changes in the moisture content of the kernels in this experiment may have been due to the fact that the cans were not sealed. In contrast, none of the cottonseed samples, which were stored in cans sealed with paraffin or in mason jars, showed any appreciable change in the moisture content during storage at the various temperatures.

TABLE 4.—*Sugar content of the kernels after storage of cottonseed at room temperature*

Analysis No.	Variety	Place grown	Date of picking	Free fatty acid content at start of test	Reducing sugars (dry basis)		Raffinose (dry basis)		Total sugars (dry basis)	
					At start of test	24 months, 25°–28° C.	At start of test	24 months, 25°–28° C.	At start of test	24 months, 25°–28° C.
				Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
A890	Farm Relief (strain 3).	Tifton, Ga.	Aug. 21, 1941	0 3	0 3	0 2	7 6	7.6	7 8	7 0
A893	Coker 100 (strain 3).	Florence, S. C.	Sept. 4, 1941	3	.4	2	7 8	8 6	8 2	8 0
A932	do	Stoneville, Miss.	Sept. 26, 1941	.2	.3	2	7 8	7 2	8 4	7 4
A935	Farm Relief (strain 3).	do	Sept. 12, 1941	4	3	2	7 7	8 1	8 3	7.8
AB201	Stoneville 37	Baton Rouge, La.	Sept. 10, 1941	30 6	.1	0	3 3	4.0	3. 5	3.7
AB203	Coker 100 (strain 3).	do	do	35 0	1	0	2 4	2. 5	3.1	3 2

TABLE 5.—*Effect of storage of cottonseed at various temperatures on its chemical composition*

Analysis No	Variety	Place grown	Date of picking	Total nitrogen (dry basis)				Oil (dry basis)				Free fatty acid content of oil			
				At start of test	14 months, 25°–28° C.	14 months, 1° C.	14 months, −18° C.	At start of test	14 months, 25°–28° C.	14 months, 1° C.	14 months, −18° C.	At start of test	14 months, 25°–28° C.	14 months, 1° C.	14 months, −18° C.
				Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
AG189	Acala	Tifton, Ga.	Sept. 2 1942	3.71	3.71	3.72	3.77	23.1	22.4	21.6	21.7	0.9	1.4	1.1	0.8
AG192	Arkansas Green Lint	do	do	3.41	3.38	3.43	3.48	21.6	21.0	21.1	20.3	4	5	6	6
AG197	Farm Relief (strain 3)	do	Aug. 19, 1942	3.45	3.28	3.22	3.44	15.6	16.1	15.6	15.9	6	9	6	.5
AG201	Rowden 42A	do	Sept. 2, 1942	3.66	3.63	3.69	3.72	19.1	19.0	18.7	18.9	4	5	5	.5
AG217	Arkansas Green Lint	Knoxville, Tenn.	Nov. 16 1942	3.18	3.22	2.91	3.13	24.7	23.4	23.2	23.3	6	1.0	8	.7
AG220	Coker 100 (strain 3)	do	Oct. 19, 1942	3.10	2.85	2.93	2.99	19.2	19.5	18.5	18.9	2	3	3	.3
AG239	Stoneville 37	do	Nov. 16, 1942	3.14	3.15	3.02	3.09	22.1	21.8	21.3	21.3	3	8	4	.3
AG241	Acala	Greenville, Tex.	Aug. 26 1942	3.67	3.61	3.63	3.62	23.3	22.9	23.3	23.1	3	5	4	4
AG253	Farm Relief (strain 3)	do	Sept. 9, 1942	3.12	3.33	3.21	3.37	21.3	21.0	21.3	21.7	2	3	3	3
AG260	Seabrook (sea island)	do	Sept. 23, 1942	3.15	3.14	3.27	3.15	19.7	19.8	19.3	19.5	1.3	1.7	1.7	1.6
AG274	Coker 100 (strain 3)	Baton Rouge, La.	Sept. 25, 1942	3.35	3.37	3.33	3.43	18.1	17.7	17.8	16.8	2.0	3.0	2.3	2.2
AG278	Rowden 42A	do	Sept. 11, 1942	3.51	3.49	3.49	3.54	21.1	21.0	20.4	20.8	2.6	3.5	2.1	2.0
AG301	Stoneville 37	Stoneville, Miss.	Sept. 14 1942	3.38	3.35	3.51	3.48	20.6	20.3	19.6	20.3	2	2	3	2
AG305	Acala	Florence, S. C.	Sept. 3, 1942	3.46	3.56	3.48	3.47	25.6	25.5	24.8	25.6	4.1	5.2	4.0	3.8
AG308	Arkansas Green Lint	do	Aug. 20, 1942	3.02	2.97	2.82	2.79	23.7	24.0	23.8	24.2	3.8	4.8	3.7	3.1
AG313	Coker 100 (strain 3)	do	Sept. 3 1942	2.88	2.89	2.80	2.83	20.9	20.4	20.7	20.4	1.9	2.3	1.8	1.6
AG318	Farm Relief (strain 3)	do	Sept. 17, 1942	3.38	3.21	3.21	3.34	22.2	23.0	21.5	22.6	1.7	2.8	1.9	1.8
AG321	Rowden 42A	do	Sept. 3, 1942	3.19	3.27	3.20	3.18	22.8	22.2	21.9	21.5	2.0	2.3	1.7	1.6
AG327	Stoneville 37	do	do	2.95	2.88	2.89	2.87	21.8	21.9	22.1	21.6	1.3	2.0	1.4	1.4
AG328	do	do	Sept. 17, 1942	3.01	3.03	2.90	2.87	20.8	20.9	20.5	20.7	3	.5	3	3
Average				3.29	3.27	3.23	3.28	21.4	21.2	20.9	21.0	1.3	1.7	1.3	1.2

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TABLE 6.—*Total sugars content and relative protein solubility of the ether-extracted kernels after storage of cottonseed at various temperatures*

Analysis No.	Variety	Place grown	Date of picking	Free fatty acid content at start	Total sugars (dry basis)				Relative protein solubility [1]			
					At start of test	14 months, 25°–28° C.	14 months, 1° C.	14 months, −18° C.	At start of test	14 months, 25°–28° C.	14 months, 1° C.	14 months, −18° C.
				Percent	*Percent*	*Percent*	*Percent*	*Percent*				
AG 253	Farm Relief (strain 3).	Greenville, Tex.	Sept. 9, 1942	0 2	9 8	14 2	14 0	14 0	79	79	80	81
AG 260	Seabrook (sea island).	do	Sept. 23, 1942	1 3	11 2	12.4	12 2	14.4	67	71	71	70
AG 305	Acala	Florence, S. C.	Sept. 3, 1942	4 1	9 1	12 6	13 5	13 7	64	79	78	77
AG 308	Arkansas Green Lint.	do	Aug. 20, 1942	3 8	11 1	12 9	14 3	14 8	69	75	76	69
AG 313	Coker 100 (strain 3).	do	Sept. 3, 1942	1 9	10 9	13 2	14 8	14.5	72	75	78	74
AG 321	Rowden 42A	do	do	2 0	10 7	13 4	14 3	14 6	76	78	79	79
AG 328	Stoneville 37	do	Sept. 17, 1942	.3	13 1	12 1	14 7	15 0	75	79	77	79

[1] Percentage of total nitrogen peptized by 0 5 N NaCl solution.

TABLE 7.— *Relative protein solubility of the ether-extracted kernels after storage of cottonseed and cottonseed meal at room temperature*

Analysis No	Variety	Place grown	Date of picking	Free fatty acid content at start of test	Relative protein solubility [1]		
					Start of test	Seed stored 14 months at 25°-28° C	Meal stored 14 months at 25°-28° C.
				Percent			
AG 269	Arkansas Green Lint	Baton Rouge, La	Aug 28, 1942	6 5	76	74	74
AG 272	Coker 100 (strain 3)	do	do	21 0	66	65	63
AG 281	Stoneville 37	do	do	19 9	68	64	65
AG 316	Farm Relief (strain 3)	Florence, S C	Aug 20, 1942	22 5	70	68	67
AG 324	Seabrook (sea island)	do	Sept 3, 1942	3 3	57	74	55

[1] Percentage of total nitrogen peptized by 0 5 N NaCl solution

TABLE 8 — *Analyses of the oil in the kernels after storage [1] of unshelled peanuts at various temperatures*

Analysis No	Variety	Free fatty acid content of oil				Iodine No of oil (Wijs)			
		At start of test	30 months, 25°-28° C	30 months, 1° C	30 months, −18° C	At start of test	30 months, 25°-28° C	30 months, 1° C	30 months, −18° C
		Percent	*Percent*	*Percent*	*Percent*				
AB 406	Tennessee Red	0 2	0 3	0 1	0 1	98	97	98	97
AB 407	Jumbo Virginia Runner	2	[2] 14 2	1	1	87	86	87	87
AB 408	Spanish (commercial strain)	3	6	2	2	95	95	95	95
AB 409	Spanish (Ga Agr Expt Sta)	5	5	2	5	96	96	96	95

[1] Stored in closed, but unsealed, metal cans
[2] This sample molded

TABLE 9.—*Analyses of the kernels of unshelled peanuts after storage* [1] *at various temperatures*

Analysis No.	Variety	Moisture				Total nitrogen (dry basis)				Oil (dry basis)			
		At start of test	30 months, 25°–28° C.	30 months, 1° C.	30 months, −18° C.	At start of test	30 months, 25°–28° C.	30 months, 1° C.	30 months, −18° C.	At start of test	30 months, 25°–28° C.	30 months, 1° C.	30 months, −18° C.
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
AB 406	Tennessee Red	4 7	5 8	6 2	5 9	5 74	5. 69	5 77	5. 83	48 2	48. 8	48. 6	48. 7
AB 407	Jumbo Virginia Runner	4 9	[2] 14 8	6 4	6 0	5 04	[2] 6 04	5 02	5 11	50. 3	[2] 47. 3	50. 3	50. 7
AB 408	Spanish (commercial strain)	5 0	5 8	6 8	6 0	5 17	5. 20	5 22	5. 22	48 5	49. 1	48. 9	48. 9
AB 409	Spanish (Ga. Agr. Expt. Sta.)	4. 8	7. 0	6 6	6 1	5 09	5 11	5 11	5. 16	48 5	49. 3	49. 0	49. 1

[1] Stored in closed, but unsealed, metal cans.
[2] This sample molded.

SUMMARY

Cottonseed samples may be stored for more than a year without appreciable change in total nitrogen, total oil, free fatty acid content, iodine, or peroxide number of the oil, or catalase activity of the kernels by drying to a moisture content of 8.3 percent or lower, placing in sealed containers, and storing at 1° C. or below. Under this type of storage at room temperature a small increase in the free fatty acid content of the oil occurs. Unshelled peanuts may be stored for more than 2 years in closed cans at 1° C. or below without appreciable change in the total nitrogen or oil content of the kernels, or in free fatty acid content or iodine number of the oil.

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NONPROTEIN AND CAROTENE AS AN INDEX OF PLANT ACTIVITY IN RANGE FORAGE[1]

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INTRODUCTION

The protein contained in plant tissues is combined in two general classifications of compounds; true proteins and nonprotein nitrogenous substances of simpler composition such as amides, amino acids, and their derivatives. These nonprotein nitrogen compounds, according to Maynard (*3*)[2] and Morrison (*4*), are abundant when plant growth is rapid but are less plentiful at maturity.

It is also generally agreed, as stated by Maynard (*3*), that all green parts of growing plants are rich in carotene, while mature plants contain much less. Negligible carotene values have been reported by Watkins (*6*) during the periods of dormancy. In experiments with rats Smith and Stanley (*5*) found the early-cut blue grama grass to be twice as potent as the mature grass in vitamin A, and 100 times as potent as the November cuttings.

It is generally accepted that the percentages of nonprotein nitrogen and carotene in plants are higher during growth than during dormancy, but just how much higher has not been known. In particular, there has been very little information concerning the amount of nonprotein nitrogen present in some of the range grasses and browse plants during the various stages of growth and maturity. This paper reports the results of monthly and regular sampling of various range grasses and browse plants of southern New Mexico covering a period of 65 months, together with nonprotein nitrogen values for 11 of the principal species of grasses and 2 browse plants; carotene values are reported for 2 of the principal grasses.

EXPERIMENTAL PROCEDURE

The arrangement of the plots and the system of collection of the range forages have been reported in detail by Watkins (*7*). The grass was harvested to a height of 2½ or 3 inches from the ground depending upon the species. The January samples include all growth produced since the preceding January. Likewise the August samples include all growth produced since the previous August. The duration of this study was 65 months. One portion of the sample was taken immediately for carotene analysis while the other portion was placed in canvas sacks, weighed, and kept in a drying oven for 7 days at a

[1] Received for publication August 3, 1945.

[2] Italic numbers in parentheses refer to Literature Cited p. 69.

temperature of approximately 60° C. It was then placed in an unheated conditioning oven for another 7 days, after which it was pulverized in a Wiley mill and placed in glass-stoppered sample bottles for analysis. This plan was rigidly followed for all samples.

The total nitrogen and the albuminoid nitrogen were determined on the various forages by the methods of the Association of Official Agricultural Chemists (*2*). The nonprotein nitrogen values are the total nitrogen less the albuminoid nitrogen. These three different nitrogen systems were converted to protein by the use of factors. The total nitrogen was changed to crude protein by using the factor 6.25, and the albuminoid nitrogen was converted to true protein by using the same factor. The nonprotein nitrogen was converted to nonprotein by using the factor 4.7 as suggested by Armsby (*1*). Hereafter in this paper the data and discussion will be with the various proteins rather than with the various nitrogen systems.

The carotene was analyzed according to the method of the Association of Official Agricultural Chemists (*2*) with slight modifications (*6*). Briefly, the procedure consists of subjecting the pulverized sample to saponification in aldehyde-free alcholic potassium hydroxide and extracting with peroxide-free ethyl ether. The ether solution, after washing free from chlorophyllins, flavones, and alkali, is distilled under reduced pressure, the residue taken up in petroleum ether (boiling point 35°–60° C.), and the xanthophyll removed by washing with 92-percent methanol.

The most important range grasses of southern New Mexico, which were studied intensively and sampled monthly, are black grama (*Bouteloua eriopoda*) and mesa dropseed (*Sporobolus flexuosus*). These were harvested from plots on the experimental ranch of the New Mexico College of Agriculture and Mechanic Arts, which lies at an altitude of approximately 4,200 feet above sea level and has an average rainfall of 9.3 inches, of which slightly more than 50 percent usually occurs in July, August, and September. This is normally the season of comparatively rapid plant growth.

Vince-mesquite grass (*Panicum obtusum*), alkali sacaton (*Sporobolus airoides*), and side-oats grama (*Bouteloua curtipendula*) were harvested and sampled every 60 days, while saltgrass (*Distichlis stricta*), bush muhly (*Muhlenbergia porteri*), chamiza (*Atriplex canescens*), and sand sagebrush (*Artemisia filifolia*) were harvested and sampled every 90 days. These forages were collected from the experimental ranch.

Range forages were also collected from plots near Estancia in central New Mexico, where the altitude is approximately 6,140 feet above sea level. Average annual precipitation is about 13 inches. Maximum temperatures in this vicinity seldom exceed 98° or 99°, but minimum temperatures of 10° to 20° below zero are not uncommon. The forages were collected four times a year from this area and included blue grama (*Bouteloua gracilis*), slender wheatgrass (*Agropyron pauciflorum*), crested wheatgrass (*A. cristatum*), smooth bromegrass (*Bromus inermis*), saltgrass, alkali sacaton, and chamiza.

EXPERIMENTAL RESULTS

Two range grasses which were studied rather intensively were mesa dropseed and black grama. In addition to the monthly harvest, other

samples were secured at various times during the growing season. The data for these two grasses, which include average results for crude protein, nonprotein, and carotene over a period of 5½ years, are presented in table 1.

TABLE 1.—*Average monthly crude protein, nonprotein, and carotene values for mesa dropseed and black grama over a period of 65 months*

Time collected	Mesa dropseed				Black grama			
	Number of analyses	Crude protein	Non-protein	Carotene	Number of analyses	Crude protein	Non protein	Carotene
		Percent	*Percent*	*Milligrams* [1]		*Percent*	*Percent*	*Milligrams* [1]
January	5	4 48	0 59	1 3	5	5 01	1 01	23 6
February	5	4 40	53	1 2	5	4 85	1 10	13 3
March	5	3 86	63	1 3	5	4 74	93	13 3
April	5	3 82	.45	11 1	5	5 10	1 00	22 7
May	5	5 59	1. 10	23 0	5	5 16	1 02	26 6
June	5	5 40	1 02	39 3	5	4 73	95	10 1
July	5	6 93	1 06	61 2	5	7 05	1 17	44 7
August	6	8 34	1 66	70 6	6	6 85	1 51	61 3
September	6	10 07	2 23	60 1	6	8 73	1 78	85 6
October	6	7 74	1 35	30 9	6	7 13	1 43	60 7
November	6	5 94	84	3 7	6	5 51	1 21	41 9
December	6	4 89	64	1 1	6	5 35	1 10	23 1
30-day growth	11	12 48	2 61		11	10 10	2 24	
15-day growth	13	13 04	2 53		11	11 20	2 44	
Beginning of growth	5	11 57	2 05		4	10 64	1 95	
End of growth	3	8 36	1 77		4	6 73	1 53	

[1] Milligrams of carotene per kilogram of feed, dry basis.

The samples designated by "30-day growth" were from the series which were collected every 30 days during the growing period; those designated "15-day growth" were collected every 15 days during the growing period. These two series represented material from immature but actively growing grass. The "Beginning of growth" samples were taken once a year at the beginning of the growing season when the grass had attained a height of 5 or 6 inches. The samples collected at the end of growth were a separate series and were taken as soon as frost occurred.

A comparison of the nonprotein, crude protein, and carotene content of mesa dropseed will indicate at least the general pattern of growth habit of this plant. Crude protein values were highest during September, which shows this to be the period of most vigorous growth. If the carotene values are considered as an index of plant activity, the mesa dropseed was active from April to November. Usually the mesa dropseed grass dies virtually to the ground with the advent of freezing weather the last of October or the first of November. Mere traces of carotene were found in dormant mesa dropseed plants during December, January, February, and March. If the nonprotein values are studied during these months when the grass is dormant, it will be seen that they were rather low, averaging approximately 0.61 percent. Values of over 1.00 percent were obtained from May to October inclusive. Plants harvested during the period of most active growth had nonprotein values of from 2.05 to 2.61 percent; thus, high nonprotein values were directly related to vigorous growth. The nonprotein percentages of the grasses collected every 15 or 30 days during

growth are among the highest values obtained with plants other than browse and apparently represent active growth rather than mere activity.

Black grama grows on the mesas, hills, and dry open ground of large areas of range land in Texas, New Mexico, Arizona, and southern Utah. The upright stems of this grass remain green for a distance of 4 to 6 inches from their bases throughout the winter. A comparison of the crude protein of black grama with that of mesa dropseed in table 1 reveals higher values for the mesa dropseed during the growing season, but higher values for black grama during the so-called dormant season. Carotene is present in black grama throughout the year. During the usual months of dormancy there are significant amounts of carotene that may in many cases provide for the requirements of beef cattle during the gestation and lactation periods (*6* and *7*). These winter carotene values are accompanied by winter plant activity. All the average monthly nonprotein values of black grama were above or only slightly below 1.00 percent. The analyses of this grass revealed 11 instances of low nonprotein values. The average of these 11 values was 0.66 percent and may represent the nonprotein content of black grama in nearly complete dormancy. This quantity is very similar to that obtained for mesa dropseed during its dormant period. If this figure of 0.66 percent is subtracted from the average nonprotein of black grama for each month, the remaining nonprotein value seems to accompany certain plant activity. In black grama this plant activity continues throughout the year, although at a much reduced rate.

The carotene and nonprotein values for the mesa dropseed and black grama grasses presented in table 1 agree in showing plant activity. When the simple correlation was calculated between carotene and nonprotein, a coefficient of 0.625 percent was obtained for black grama and 0.48 percent for mesa dropseed (table 2). These correlations are highly significant and seem to establish the fact that nonprotein values as well as carotene may be used in estimating plant activity. Although there is a relation between these numerical values and plant activity, the proportion does not appear to be direct. The nonprotein and carotene data for each of the two grasses have been averaged by months and are presented in figure 1 for comparison.

TABLE 2.—*Correlation of nonprotein (x) and carotene (y) in black grama and mesa dropseed*

Grass	Number of comparisons	Sum of squares and products			Correlation coefficient
		Sx^2	Sxy	Sy^2	
Black grama	59	8.34	411.06	52,064.4	0.625*
Mesa dropseed	59	22.44	634.30	76,769.8	.48*

*Highly significant.

A number of other grasses and forage plants were studied during regular but less frequent intervals. It is obvious that with 60- or 90-day sampling, the data are necessarily incomplete. However, there are observations which are worthy of attention. In studying the

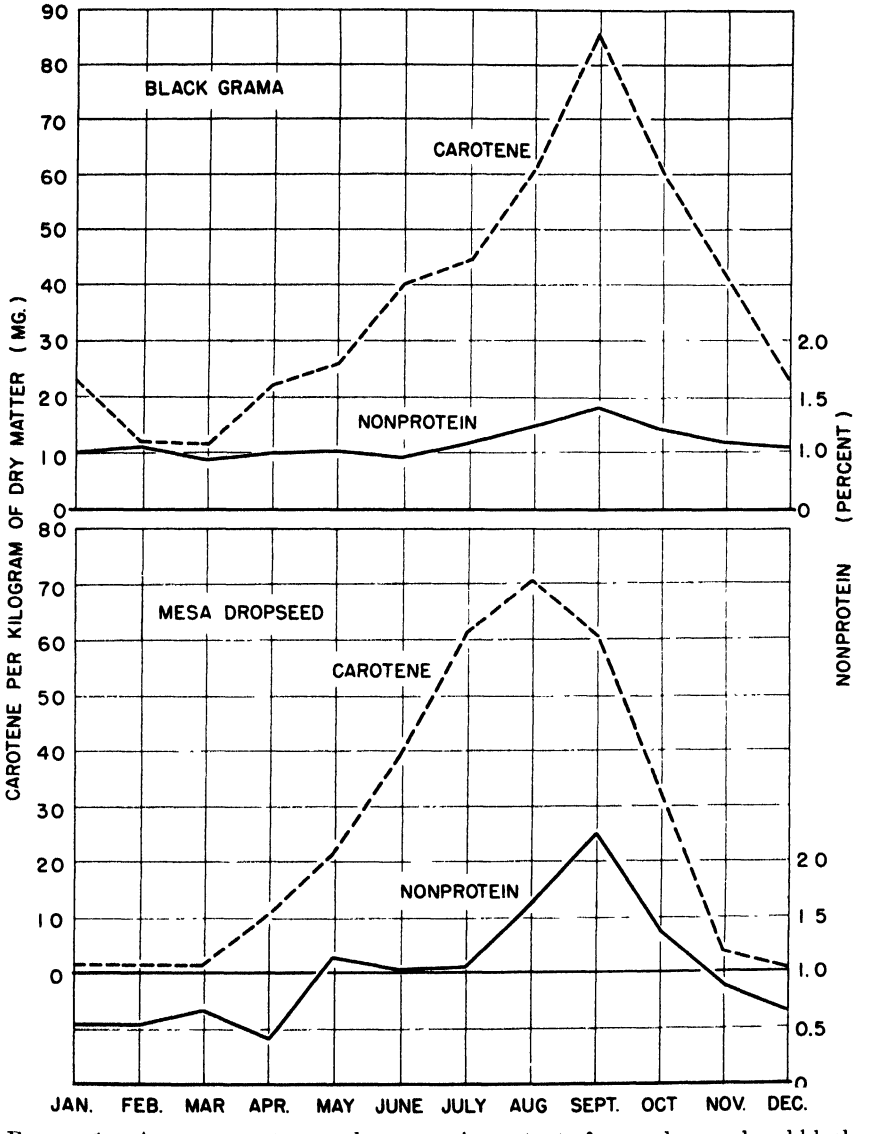

FIGURE 1.—Average carotene and nonprotein content of mesa dropseed and black grama covering 59 months.

nonprotein values for various grasses and browse plants, which are presented in table 3, it will be assumed, as stated earlier in this paper, that a nonprotein value of over 0.66 percent represents an increment of plant activity. The value of 0.66 percent seems satisfactory for black grama, but may be slightly high for the dormant period of some of the other grasses as is indicated by their February and March values. Since this seems to be true with this limited amount of data,

the nonprotein percentages of 0.66 or above readily become significant in determining plant activity. Most of the grasses are above 0.66 percent in nonprotein from May to October with the period of most active growth occurring within this time. Bush muhly grass is an exception and has moderately high nonprotein values which remain constant for each sampling time. This grass is similar to black grama in that it remains partly green throughout the year.

TABLE 3.—*Percentage of nonprotein in various grasses and browse collected at 60- or 90-day intervals*

Grasses	Number of analyses	February	March	April	May	June	August	September	October	November	December
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
From the college ranch plots in southern New Mexico:											
Vine-mesquite	32	0 29		0 55		1 02	1 49		1 02		0 31
Alkali sacaton	32	.48		68		97	1 81		1 45		67
Side-oats grama	32	27		31		53	1 35		97		23
Saltgrass	22	50			2 10		1 03			1 11	
Bush muhly	21	1 34			1 40		1 34			1 34	
Chamiza	22	2. 71			3 62		4 93			3 56	
Sand sagebrush	22	1. 02			1 14		2 42			1 61	
From the Estancia plots in central New Mexico:											
Blue grama	16		0 30			1 16		1 44			.41
Slender wheatgrass	16		49			2 38		2 08			63
Crested wheatgrass	16		1 52			2 02		2 01			73
Smooth bromegrass	16		3 13			2 26		2 20			86
Saltgrass	16		49			2 58		1 71			65
Alkali sacaton	16		50			3 02		2 29			78
Chamiza	15		3 05			4 51		3 33			1 33

Chamiso, an evergreen browse plant, has a high percentage of nonprotein. Results from Estancia in central New Mexico showed that the percentages of nonprotein for chamiza were lowest in December. Since winter temperatures of 10° to 20° below zero are common in this area, it is possible that during the winter this plant has much less activity in central New Mexico than in the southern part of the State. Sand sagebrush is another evergreen browse plant with pronounced growth during the growing season but with much less activity during the rest of the year. Crested wheatgrass and smooth bromegrass have significant high nonprotein values from March through September. Field observations also confirm the fact that these two grasses are very early starting in the spring. This is one of the reasons why they are being used for pasture reseeding purposes at some of the higher altitudes.

The botanist and plant breeder may make use of this nonprotein as an aid to field and other observations, if, when planning a project, the albuminoid nitrogen as well as the total nitrogen is to be determined upon the plant material.

SUMMARY

The percentages of nonprotein in various New Mexico range forages are presented. Some of the range grasses and browse plants have

continuous plant activity throughout the winter, though the rate of activity in certain species is much reduced. The percentages of nonprotein for black grama and mesa dropseed grasses are closely correlated with the carotene level. Analyses of the nonprotein, as well as of the crude protein and carotene values when these were available, together with field observations, formed the basis for estimating the plant activity of these forages.

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ALTERNARIA BLIGHT AND SEED INFECTION, A CAUSE OF LOW GERMINATION IN CERTAIN RADISH SEED CROPS[1]

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INTRODUCTION

The production of radish seed is an important agricultural specialty in certain areas in Michigan. Seed crops usually are grown by farmers on a contract basis for various seedsmen and harvested seed must meet a minimum germination standard of about 85 percent. Seed crops which show substandard germination as specified by the seedman's tests frequently are discarded with a loss to the growers.

Seed crops of substandard germination generally occur in seasons of excessive rainfall subsequent to harvest, and it is during such seasons that radish plants usually are seriously blighted by infections of the *Alternaria* fungus. The disease is present in a greater or lesser degree every year.

In 1944 a project was initiated at the Michigan Agricultural Experiment Station to study the causes of low germination of radish seed crops. In a series of experiments Barrons and McLean[2] showed that the occurrence of low-germinating radish seeds may be influenced by high moisture in the storage bag after threshing. The growth of parasitic and saprophytic organisms was not correlated with high moisture during storage. In another experiment with samples of seeds from low-germinating lots they found approximately 40 percent infection by species of *Alternaria*. Four species of *Alternaria* were isolated from infected seeds harvested from a single planting.

Further studies were made in the field and in the laboratory during the season of 1945 to correlate pod infections by *Alternaria* with seed infections as a cause of low germination. This article records the results.

SYMPTOMS OF THE DISEASE

Since several species of *Alternaria* have been isolated from radish seeds (*Raphanus sativus* L.) taken directly from diseased pods, it is assumed that each organism must have invaded the seed through

[1] Received for publication May 22, 1946. Journal article No. 820 (n.s.) from the Michigan Agricultural Experiment Station.

[2] BARRONS, K. C., and MCLEAN, D. M. A STUDY OF THE CAUSES OF LOW GERMINATION OF RADISH SEED CROPS. Mich. Agr. Expt. Sta. Quart. Bul 27: 398–408. 1945.

FIGURE 1.—Lesions on radish seed pods caused by *Alternaria raphani*.

the pods. No attempt has yet been made to determine the symptoms of the infections caused by each species. From a great number of isolations made from leaf, stem, pod, and seed infections *Alternaria raphani* Groves and Skolko has been observed. The symptoms, as they are described here, are those caused by infections of this fungus in inoculation experiments.

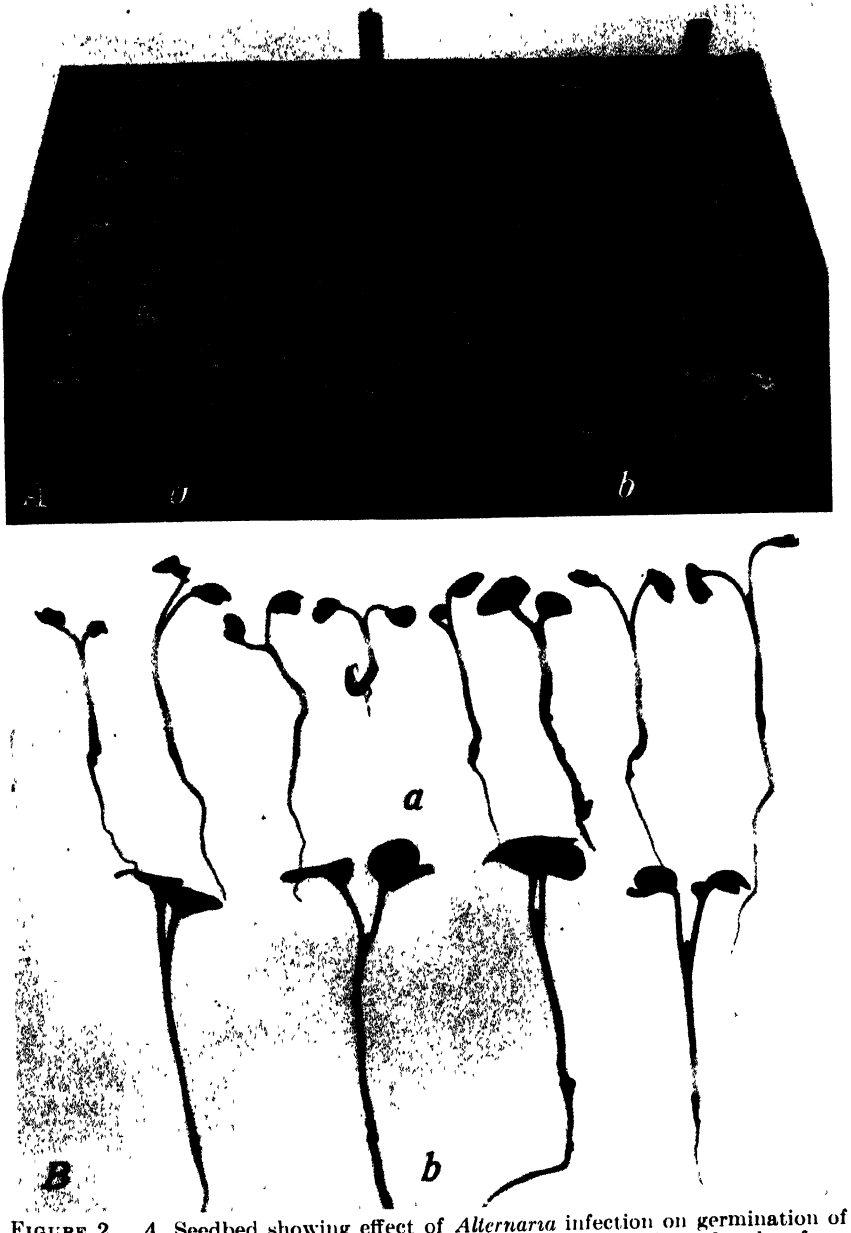

FIGURE 2. *A*, Seedbed showing effect of *Alternaria* infection on germination of radish seeds; *a*, seeds taken from apparently healthy pods; *b*, seeds taken from diseased pods. *B*, Radish seedlings: *a*, Infected by *A. raphani*; *b*, healthy.

Infections first become evident on the leaves of the fruiting stalk and may be found from the basal leaves to the tip leaves. The lesions first appear as very small yellowish-colored, slightly raised

spots one-half millimeter or less in diameter. With age, the lesions enlarge to approximately a centimeter and become roughly spherical to broaden elliptical. The centers of the older spots are of a thin, translucent, papery consistency usually accompanied by an olivaceous to grayish or blackish growth of the fruiting fungus. On older leaves the centers of the lesions may drop out leaving a "shot-holed" appearance. Individual lesions usually are bordered by a slightly raised margin or ridge. The fungus generally sporulates abundantly on the foliar lesions.

Before the seeds are mature, early pod and stem infections are visible as purplish to brownish irregular lesions (fig. 1). During periods of abundant rainfall, infections are so severe that entire pods may be included in the lesion. Usually, the stylar end of the infected pods is blackish and shriveled. When mature, the infected pods are often covered with blackish lesions from pin-point size to a centimeter or more (figs. 1 and 4, *B*, *a*). Only rarely are spores found on the pod lesions; however, some pods may be covered with a mass of olivaceous to blackish-colored spores. The fungus may be readily isolated from pod infections. Stem infections are common and the lesions resemble those on the pods (fig. 1). Infections may occur on exposed parts of the root crown, causing dark circular lesions.

The fungus penetrates deep into the pod tissues and may infect the immature seeds in the pods. Where young pods are severely infected the seeds may not develop. In older pods, the seeds may be shriveled and show darkened areas on the seed coats (fig. 4, *B*, *a*). Infected seeds may not germinate or pre-emergence or postemergence damping-off of seedlings may occur (fig. 2, *A*, *B*). Any portion of the seedling may be attacked. The fungus may readily be isolated from diseased seedlings.

SPECIES OF ALTERNARIA ISOLATED FROM RADISH SEEDS IN MICHIGAN

Four species of *Alternaria* have been isolated from radish seeds taken from crops grown in Michigan (fig. 3, *A–D*). Groves and Skolko [3] determined seven species of *Alternaria*, of which six are definitely pathogenic, from a great number of agricultural seeds collected from nearly world-wide sources. These writers have very adequately described the species found in the present investigation [3]. Of the four species found infecting radish seeds *A. raphani* is the most common and is extremely pathogenic. *A. brassicae* and *A. oleracea*, which are pathogenic to seedlings of various cruciferous hosts,[3] have been observed in only a few instances. *A. tenuis* includes the generally considered saprophytic forms and is common in plate cultures from radish seeds.

It is not the purpose of this paper to go into a detailed discussion of nomenclature. Elliott,[4] Wiltshire,[5] and Groves and Skolko [3] have

[3] GROVES, J. W., and SKOLKO, A. J. NOTES ON SEED-BORNE FUNGI. II. ALTERNARIA. Canad. Jour. Res. Sect. C 22: 217–234, illus. 1944.

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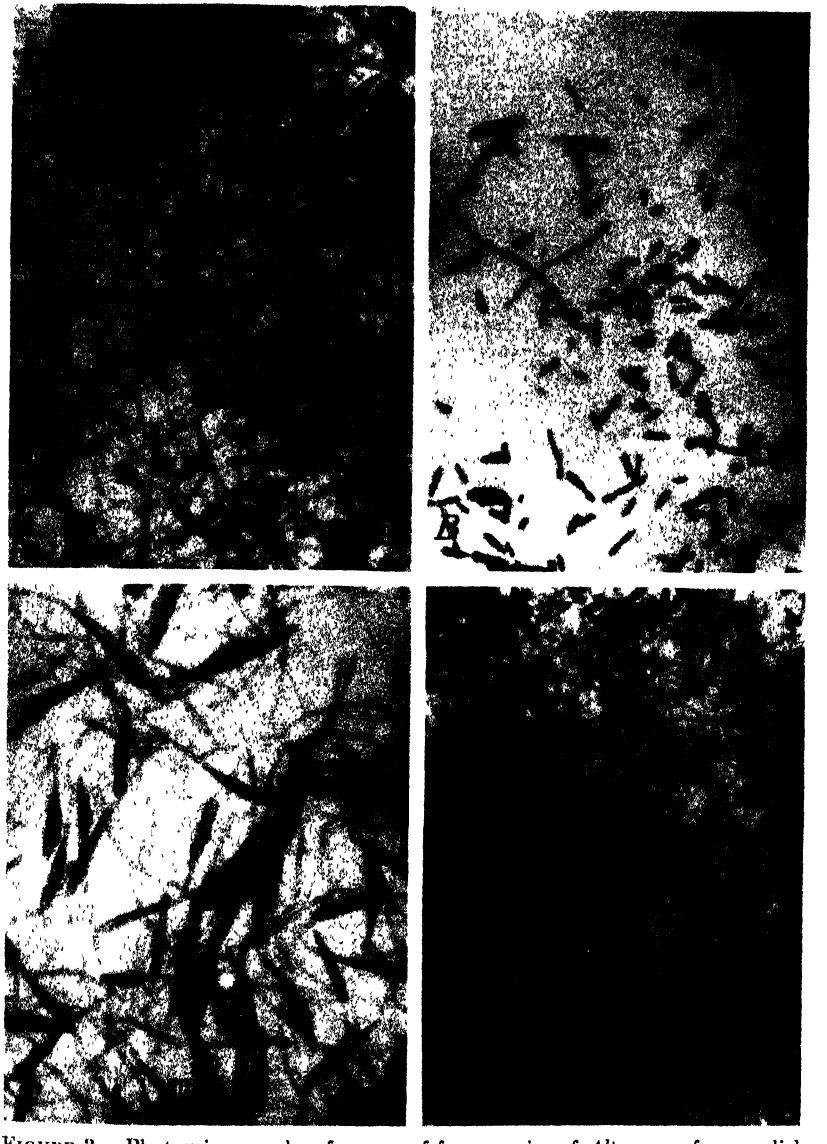

FIGURE 3.—Photomicrographs of spores of four species of *Alternaria* from radish seeds: *A*, *A. raphani*; *B*, *A. oleracea*; *C*, *A. brassicae*; *D*, *A. tenuis*.

thoroughly discussed the genus *Alternaria*. The identification of the species found in this investigation has been verified by Dr. J. W Groves.[6]

[6] The writer acknowledges his indebtedness to Dr. Groves, of the Central Experimental Farm, Department of Agriculture Ottawa, Canada, for the verification of the species here discussed.

EXPERIMENTAL METHODS AND RESULTS OBTAINED

In a preliminary series of experiments conducted in 1944, it was found that a relatively high percentage of seeds of low-germinating seed lots were infected by parasitic and saprophytic organisms. Seed samples from a number of radish crops showing germination of 40 to 80 percent were obtained from seedsmen and growers in the State. Seeds from each sample were surface-sterilized by dipping them in 1 : 1,000 corrosive sublimate for 10 minutes after which they were rinsed in sterile water and placed on a potato-dextrose-agar medium in Petri plates. After several days, the *Alternaria* fungus was growing from as many as 40 percent of the seeds in certain samples. The counts of infected seeds were made without attempting to determine the relative number of species. *Fusarium*, *Penicillium*, *Rhizopus*, and certain bacteria were present in occasional instances, but these were not considered a principal cause of low germination. *A. raphani* was the organism most commonly found on seed samples of low vitality. Groves and Skolko also found species of *Alternaria* to be the most common organisms on low-germinating radish seeds in Ontario, Canada.

Seed treatment with the common dust materials did not significantly increase germination in the low-germinating samples. The same result was obtained by Groves and Skolko.[7] Infected seeds treated in a water bath at 50° C. (122° F.) for 25 minutes germinated free of *Alternaria* on agar media. Hot water treatment, however, did not increase germination percentages in low-germinating diseased seeds. It seemed evident that whatever the cause of low germination it was operative before the seeds were marketed or even before they were harvested. With this point in mind, further experimentation was conducted in 1945.

Diseased plants with mature pods were collected in the field and brought to the laboratory, where they were thoroughly dried. Seeds from individual diseased pods showing numerous lesions and seeds from individual pods apparently free from infection were placed on potato-dextrose agar in Petri plates in the same position that they occupied in the pods. These seeds were not surface-sterilized, but were transferred directly from the pods to the Petri plates. After several days, seeds from pods with numerous lesions showed 75 to 80 percent infection with *Alternaria*. Often all the seeds in the diseased pods were infected (fig. 4, *B*, *b*). Usually, the seeds directly beneath the pod lesions were infected and frequently the seed coat showed dark markings. Seeds from pods which had only an occasional lesion, the majority of which appeared healthy, likewise frequently showed 10 to 15 percent infection. *A. raphani* was the only fungus observed in this experiment.

In another experiment, seeds were taken from diseased pods and from apparently healthy pods for testing germination (fig. 4, *A*, *a* and *b*). Seeds taken from diseased pods were slightly smaller than those from apparently healthy pods and many showed dark markings on the seed coat. Seeds which were shriveled and those which proba-

[7] See footnote 3, p. 74.

FIGURE 4. *A*, *a*, Radish seeds taken from pods infected with *Alternaria*, showing dark areas on seed coats; *b*, seeds taken from apparently healthy pods. *B*, *a*, Diseased radish pods; *b*, *A*, *raphani* growing on seeds taken from diseased pods and placed on potato-dextrose agar.

bly would be eliminated in the cleaning process after threshing were removed from the lot. Samples of 500 seeds taken from diseased pods and from apparently healthy pods were planted in sterilized sand. After 9 days the number of seedlings were counted in each case. The results are presented in table 1.

TABLE 1.—*Germination percentages of radish seeds taken from diseased pods and from apparently healthy pods*

Block No.	Seeds from diseased pods		Seedlings infected	Seeds from apparently healthy pods		
	Planted	Germinated		Planted	Germinated	Seedlings infected
	Number	*Percent*	*Percent*	*Number*	*Percent*	*Percent*
1	100	36	9	100	90	13
2	200	27	10	200	87	14
3	200	35	11.5	200	81	14.5
4	500	31		500	85	
5	500	32		500	85	

The data in table 1 show (1) that *Alternaria* infection causes a marked reduction in germination of seeds harvested from diseased pods, and (2) that seed taken from apparently healthy pods in a diseased planting may be infected even though there is little evidence of pod infection. The experiment was repeated several times with similar results. *A. raphani* was the only fungus isolated from the diseased seedlings.

Lesions were observed on the cotyledons, hypocotyl, and roots of diseased seedlings, and infected seedlings frequently damped off (fig. 2 *B*, *a*).

It was during the course of this experiment that cultural variations were noticed in the different *Alternaria* isolates from infected seeds. A detailed study of the different isolates led to the identification of the species previously mentioned. *A raphani* was the species most commonly observed.

DISCUSSION

The results of the foregoing experiments have shown that infection by *Alternaria* is a principal cause of low germination in radish seed of certain crops. Groves and Skolko [8] found a direct correlation between germination and the presence of *A. raphani* in seed samples in which germination ranged from 14 to 65 percent. This species was the only pathogenic fungus that appeared consistently in all their samples in a high percentage. The organisms other than *Alternaria* which are frequently isolated from radish seeds are probably unimportant as a cause of low germination. For the most part they appear to be saprophytic and occur during the curing process before the seeds are dry. High moisture during storage may cause increased infections by the *Alternaria* fungus. When pod infections occur, the spores are mixed with the seed during threshing, and the mycelium may continue to infect the seeds and give a moldy appearance to the crop.

Since it has been shown that percentage germination of seeds from diseased pods is lower than that of seeds from apparently healthy pods, it appears that low germination may be correlated directly with pod infections in the field. This seems to be indicated also by the periodicity of good and bad seed crops in Michigan. Radish plantings for seed crops are blighted with *Alternaria* infections nearly

[8] See footnote 3, p. 74.

every year in Michigan. In certain seasons crops are harvested which give high germination percentages, while in others germination is low. Low germination percentages are usually found in seasons of excessive rainfall when *Alternaria* infection is serious. Various reasons have been given to account for low germination during these years, including (1) poor pollination, (2) wet weather during the curing period, (3) excessive moisture after sacking from the threshing machine, and (4) the prevalence of seed-borne pathogens. Some of these reasons are correlated with conditions which favor infection by *Alternaria.*

Since neither fungicidal seed treatments nor hot water disinfection of diseased seeds necessarily induces a higher germination of infected seed, control measures must be applied in the field. Inasmuch as *Alternaria raphani* seems to be the principal cause of the disease and apparently has been isolated only from radish, it follows that if disease-free seed were planted the severity of seed infection might be controlled by destruction of the source of primary inoculum.

SUMMARY

Four species of *Alternaria* have been isolated from low-germinating radish seeds in Michigan. *A. raphani* causes infections of the leaves, stems, pods, and roots of radish plants and is the most common parasitic species that has been isolated. Seeds taken from infected pods frequently show 70 to 80 percent infection. This species is also pathogenic to seedlings. *A. raphani* is considered to be the cause of infection in the field which results in low germination in infected seed crops. *A. brassicae* and *A. oleracea* are found only rarely and probably are not of primary importance as a cause of low germination in seed crops. *A. tenuis* is a type commonly found on radish seeds, but it is considered to be mostly saprophytic. *Penicillium*, *Rhizopus*, *Fusarium*, and certain bacteria frequently are found in seeds after the curing process.

Fungicidal dust treatments of low-germinating seeds have failed to give significant increases in percentage of seedling counts. Hot water treatment for 25 minutes at 50° C. (122° F.) killed the *Alternaria* pathogen in infected seeds. Such treatment, however, did not increase germination precentages in low-germinating seed lots.

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GROWTH RESPONSES TO ORGANIC COMPOUNDS BY TOBACCO SEEDLINGS IN ASEPTIC CULTURE[1]

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INTRODUCTION

Investigations on the responses of plants to organic compounds of natural and synthetic origin have recently tended to assume considerable importance. The increased knowledge of the biochemistry of plants thus obtained has been utilized for making more practical the methods of producing mutants, rooting cuttings, and preventing fruit drop and for improving materials for the protection of plants against micro-organisms and insects. On the other hand, soil toxicity due to organic substances has been shown to exist (*7*).[2] The action of crop residues on the growth of subsequent crops (*3*) also may be partially of similar origin. Knudson (*4*) and others demonstrated that sugars and other compounds may be absorbed by the plant through its roots and lead to increased growth. No necessity exists, therefore, to justify the further use of this experimental procedure.

The studies dealt with in this paper originated when the observation was made that seedlings of the Robinson strain of Maryland Medium Broadleaf tobacco grown in aseptic culture under low-intensity artificial light at constant temperature did not pass through the rosette stage on an 8-hour day. The attempt to eliminate this formative effect of light led to a study of the influence of organic compounds of natural origin on growth. Though most of these data are for tobacco seedlings grown with a short day, it is believed that they do not differ in any important degree from those obtainable for plants grown with a long day.

MATERIALS AND METHODS

Seedlings of Maryland Medium Broadleaf tobacco were grown aseptically in 200-cc. Pyrex Erlenmeyer flasks containing 50 cc. of nutrient solution solidified with agar. The solution used was that developed by McMurtrey (*6*), and the 20 × solution contained water, 1,000 cc.; $Ca(NO_3)_2.4H_2O$, 29.16 gm.; KNO_3, 2.19 gm.; $Mg(NO_3).6H_2O$, 3.19 gm.; KH_2PO_4, 5.75 gm.; $MgSO_4.7H_2O$, 3.08 gm.; and NH_4Cl, 1.50 gm. The micronutrients in this concentrated solution

[1] Received for publication August 23, 1946.

[2] Italic numbers in parentheses refer to Literature Cited, p. 91.

comprised zinc, 10.0 mg.; copper, 2.5 mg.; manganese, 20.0 mg.; and boron, 10.0 mg. Iron was added separately to the diluted solution as ferric chloride at a concentration of 3 p. p. m. After dilution of this solution and addition of iron, 2 percent of sucrose, and 1.15 percent of agar, the medium was heated and put in flasks, which were then plugged with absorbent cotton and sterilized at 15 pounds' pressure for 30 minutes. The organic compounds under test were added to the flasks before they were filled with the medium.

Seed was sterilized with 1:1,000 silver nitrate for 15 minutes, washed with three changes of distilled water, and poured into previously sterilized petri dishes containing several layers of filter paper. The seedlings were transferred to the flasks after a germination period of 6 to 7 days at 25° C. The use of a pair of magnifying spectacles (2.5 ×) was helpful. A flamed platinum hook with a sharpened 2-mm. prong was moistened with sterile distilled water and brought in contact with the seedling, and the latter was transferred to the agar. The seedling adhered first to the platinum needle and then to the agar. Seedlings with a total length of 5 mm. were readily handled, as the radicles could be pushed into the agar with the back of the prong.

The seedlings were grown at 25° C. in lighted incubators under 3,500° white fluorescent lamps furnishing 500 foot-candles of illumination for 28 days. On harvesting, the seedlings were freed from the agar, rinsed with water, pressed between absorbent paper, and dried at 103° to 105° for 4 hours. Usually the values shown are the average of four duplicate cultures. The cultures were weighed in pairs. Contaminated cultures and abnormal plants were discarded. Injury to seedlings during transfer, immersion of cotyledons, and exposure of the radicles caused marked reductions in growth. Rejections due to these causes were about 10 percent.

EFFECTS OF SUGARS ON GROWTH

Growth in a medium containing sugars under the experimental conditions used is shown in table 1. The average dry weight obtained after 28 days of growth with 8 hours of light daily was 4 mg. The seedlings formed stems immediately and four to six leaves of good color. With continuous illumination the total yield per plant averaged 68.9 mg., the leaves were normal in shape and color, and the rosette stage was just on the point of termination. The main effect of sucrose (2 percent) was on the vigor and rate of growth of the seedling; the yields became 19.7 mg. with 8 hours of daily illumination and 150.5 mg. with continuous illumination.

The effects of other sugars and compounds listed in the table were studied on seedlings grown under 8 hours of daily illumination. Only with D-glucose and D-fructose did growth increase approach that with sucrose. D-fructose, however, caused root injury. None of the other compounds tested, with the possible exception of D-xylose, caused an increase in growth.

TABLE 1.—*Growth of tobacco seedlings, Maryland Medium Broadleaf, with various sugars in aseptic culture for 28 days at 25° C. and 500 foot-candles of fluorescent white light for 8 hours daily*

Treatment or compound (20 gm. per liter)	Average total yield	Appearance [1]	Treatment or compound (20 gm. per liter)	Average total yield	Appearance [1]
	Milligrams			*Milligrams*	
Control	4.0	S, 8	L-Arabinose	1.4	S, 9
8-hour day	19.7	S, 8	D-Mannitol	2.3	S, 8
8-hour day with sucrose	68.9	R, 10	Calcium citrate	3.6	S, 8
Continuous day	150.5	R, 10	Calcium D-gluconate	3.9	S, 6
Continuous day with sucrose	15.6	S, 8	Calcium 2-keto-D-gluconate	.9	S, 4
D-Glucose	14.6	[2] S, 8	Calcium 5-keto-D-gluconate	.5	S, 4
D-Fructose	1.6	R, 8	Calcium D-lactate	.7	S, 4
D-Mannose	0	–, 0	Potato starch	1.1	S, 6
D-Galactose	.8	S, 8	Sucrose+D-mannose [3]	18.9	[2] R/2, 8
L-Sorbose	7.5	S, 8	Do [4]	11.9	[2] R, 10
D-Xylose	0	–, 4	Do [5]	6.3	[2] R, 10
D-Arabinose					

[1] S, Shoot or stem; R, rosette; R/2, partial rosette. Rated from 0 (white) to 10 (dark green).
[2] Short roots, injury.
[3] Mannose at 10 gm. per liter.
[4] Mannose at 20 gm. per liter.
[5] Mannose at 30 gm. per liter.

D-mannose appeared to be the only compound that inhibited stem formation, that is, aided passage through the rosette stage. It was not possible to eliminate premature stem formation with D-mannose, however, without decreasing yield. A mixture of 10 gm. of D-mannose and 20 gm. of sucrose was partially effective in promoting rosette formation without seriously diminishing yield.

Figure 1 illustrates the types of growth obtained on an 8-hour and a 24-hour day with and without sucrose. Growth with various sugars is shown in figure 2.

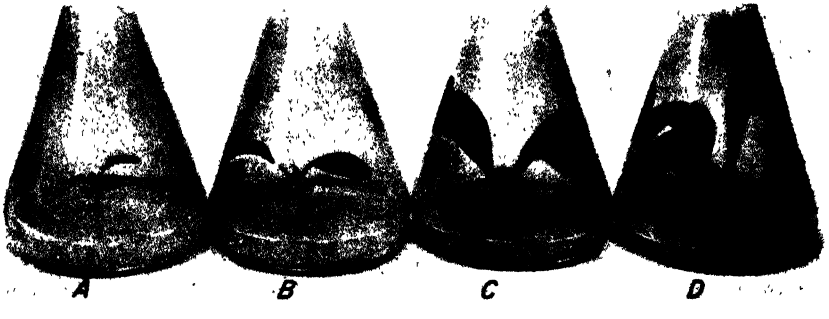

FIGURE 1.—Maryland Medium Broadleaf tobacco seedlings, 3 weeks old, grown aseptically on mineral agar at 25° C. with 500 foot-candles of fluorescent white light: *A*, With 8 hours of daily illumination but without sugar in medium; *B*, with 8 hours of daily illumination and 2 percent of sucrose in medium; *C*, with continuous illumination but without sucrose in medium; *D*, with continuous illumination and sucrose in medium.

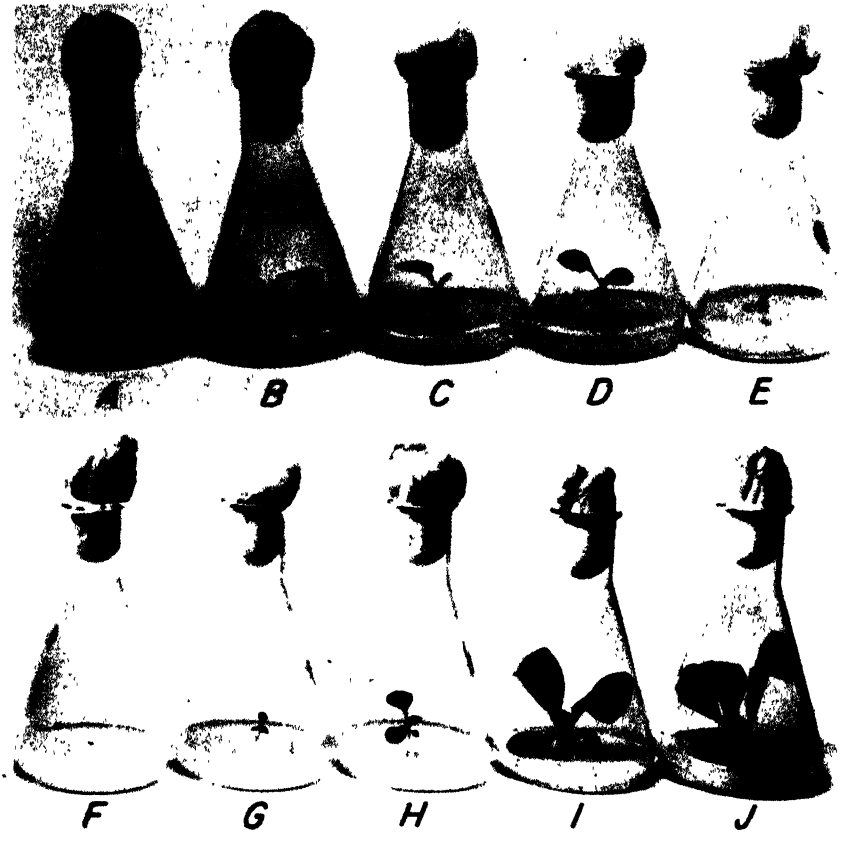

FIGURE 2.—Maryland Medium Broadleaf tobacco seedlings, 3 weeks old, grown aseptically on mineral agar at 25° C. with 500 foot-candles of fluorescent white light: *A* to *H*, With 8 hours of daily illumination—without sugar (*A*) and with 2 percent each of sucrose (*B*), D-glucose (*C*), D-fructose (*D*), D-mannose (*E*), D-galactose (*F*), L-sorbose (*G*), and D-xylose (*H*); *I* and *J*, with continuous illumination—without sucrose (*I*) and with 2 percent of sucrose (*J*).

EFFECTS OF AMINO ACIDS ON GROWTH

The results of tests with amino acids on growth of tobacco seedlings are shown in table 2. All media contained 2 percent of sucrose, and the cultures were grown on an 8-hour day. Growth was diminished almost without exception at a concentration of 200 p. p. m. of amino acid. *d*-Arginine hydrochloride, *l*-cystine, and *dl*-ornithine hydrochloride proved least toxic in the order given.

Toxicity at a level of 200 p. p. m. was associated with excessive acidity in the case of *l*-aspartic acid (pH=3.98), *d*-glutamic acid (pH=4.08), *l*-histidine dihydrochloride (pH=4.07), and *d*-lysine dihydrochloride (pH=3.99). Cultures of excessive acidity were easily recognized by failure of the agar to gel. The acidity factor, however, was probably of minor importance in itself at the above-mentioned acidities. Glycine (pH=4.96), *l*-hydroxyproline (pH=

TABLE 2.—*Growth of tobacco seedlings, Maryland Medium Broadleaf, with 2 percent of sucrose plus various amino acids in aseptic culture for 28 days at 25° C. and 500 foot-candles of fluorescent white light for 8 hours daily*

Control or compound (200 mg per liter)	Average total yield	Appearance [1]	Control or compound (200 mg. per liter)	Average total yield	Appearance [1]
	Milligrams			*Milligrams*	
Control	20.1	S, 8	*dl*-Isoleucine [2]	1.8	R, 4
dl-Alanine	.4		*d*-Lysine dihydrochloride	2.0	R, 4
dl-α-Amino-*n*-butyric acid	0		*dl*-Methionine	4.8	S, 8
d-Arginine hydrochloride	18.3	S, 8	*dl*-Norleucine	.5	R, 2
l-Asparagine	.5	R, 0	*dl*-Ornithine hydrochloride	13.9	S, 8
l-Aspartic acid	2.0	R, 6	*dl*-β-Phenylalanine	10.1	S, 8
l-Cystine	15.6	S, 8	*l*-Proline	.1	R, 2
d-Glutamic acid	.6	R, 2	*dl*-Serine	0	
Glutathione	1.4	R, 2	*dl*-Threonine	.2	R, 2
Glycine	.5	R, 1	*l*-Tryptophane	.6	R, 2
l-Histidine dihydrochloride	4.8	R, 8	*l*-Tyrosine	10.4	S, 8
l-Hydroxyproline	0	—, 0	*dl*-Valine	.3	R, 2
l-Leucine	9.2	S, 8			

[1] S, Shoot or stem; R, rosette. Rated from 0 (white) to 10 (dark green)
[2] Seedlings grown with *dl*-isoleucine showed inhibition in growth of apical bud, suckering, and strap leaves; all typical symptoms of frenching.

4.92), *dl*-isoleucine (pH=4.89), *l*-proline (pH=4.76), *dl*-serine (pH=4.78), *dl*-threonine (pH=4.78), and *l*-tryptophane (pH=5.14) were very toxic though having acidities comparable to that of the control (pH=4.51). All these acidities were measured at the time of harvest. Initial acidity of the control was equivalent to a pH of 5.11.

In no instance was shoot development inhibited by an amino acid at a concentration of 200 p. p. m. except when it checked all growth. Chlorosis when present was not distinctive as a rule but consisted in a uniform bleaching of all leaves except the cotyledons.

Marked and characteristic abnormalities occurred only in the presence of *dl*-isoleucine (*9*). The rosette condition persisted with *dl*-isoleucine because of inhibition of stem and sucker growth and profuse development of the axillary leaves. As compared with the controls, an excessive number of leaves were formed which exhibited a slight mottling and varied from narrow to "strap leaf." The general appearance of these leaves was very similar to that of leaves of tobacco plants affected with the disease known as frenching. Divergencies, of course, existed. These differences consisted in the absence of a distinctive reticular chlorosis, the presence of curved leaf margins, the absence of pale bud and leaf margins, and the presence of the characteristic waving and ruffling of the strap-leaf margins.

The appearance of seedlings grown in a medium containing *dl*-isoleucine is shown in figures 3 and 4. Strap-leaf formation was noticeable in 14 days and occurred in the first pair of leaves formed. It is improbable that these responses were associated with an impurity, for similar results were obtained with two different samples purchased several years apart from the same manufacturer and with a third sample from a second manufacturer.

Additional tests were made with the most toxic of the amino acids at a lower level of concentration (50 p. p. m.). The results are given in table 3. Most of these amino acids were rather toxic under the conditions of the tests even at these high dilutions.

TABLE 3.—*Growth of tobacco seedlings, Maryland Medium Broadleaf, with 2 percent of sucrose plus amino acids of high toxicity in aseptic culture for 28 days at 25° C. and 500 foot-candles of fluorescent white light for 8 hours daily*

Control or compound (50 mg. per liter)	Average total yield	Appearance [1]	Control or compound (50 mg. per liter)	Average total yield	Appearance [1]
	Milligrams			*Milligrams*	
Control	17.9	S, 8	*dl*-Isoleucine [3]	3.1	[4] R, 4
dl-Alanine	.2	R, 2	Do. [5]	1.7	[6] R, 2
l-Aspartic acid	9.6	S, 6	*d*-Lysine dihydrochloride	11.0	S, 6
d-Glutamic acid	.7	R, 2	*l*-Proline	4	S, 2
Glycine	4	R, 2	*dl*-Serine	3	S, 2
l-Histidine dihydrochloride	11.2	S, 6	*dl*-Threonine	1.6	S, 1
l-Hydroxyproline	.1		*l*-Tryptophane	8.8	R, 8
dl-Isoleucine	4.2	[2] R, 6	*dl*-Valine	.5	R, 1

[1] S, Shoot or stem; R, rosette. Rated from 0 (white) to 10 (dark green).
[2] Narrow leaves.
[3] 100 mg. per liter.
[4] Narrow and strap leaves.
[5] 200 mg. per liter.
[6] Strap leaves.

The frenching response with *dl*-isoleucine persisted but with diminished severity. For example, strap leaves were not formed at the lowest concentration though the widths of the leaves were decreased by more than half. It is possible, however, that a search would reveal conditions under which the action of *dl*-isoleucine would be exhibited at even a lower concentration than 50 p. p. m. It was the

FIGURE 3.—Maryland Medium Broadleaf tobacco seedlings grown aseptically at 25° C. with 500 foot-candles of fluorescent white light on mineral agar containing 2 percent of sucrose and 200 p. p. m. of *dl*-isoleucine: *A*, Seedlings 4 weeks old, grown with 8 hours daily illumination; *B*, same seedlings after being grown an additional month with continuous illumination.

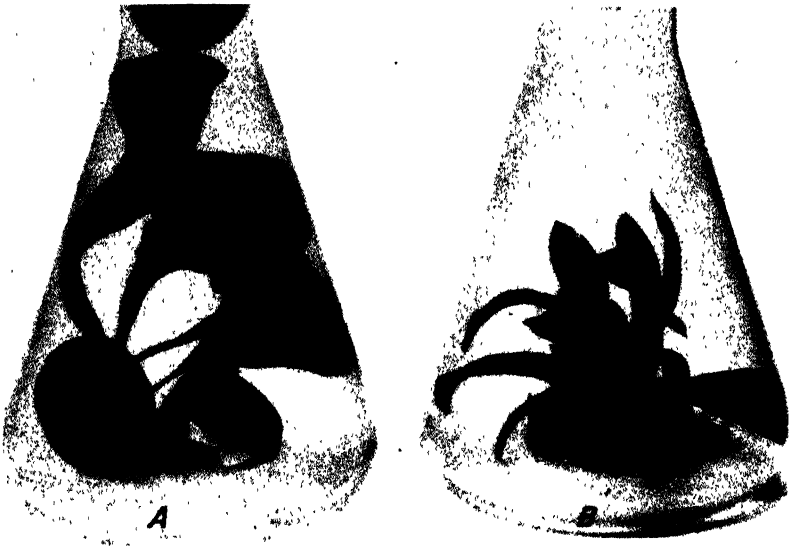

FIGURE 4.—Maryland Medium Broadleaf tobacco seedlings grown from September 5 to October 25, 1945, at 25° C. with 500 foot-candles of fluorescent white light and continuous illumination on mineral agar containing (*A*) 2 percent of sucrose and (*B*) 2 percent of sucrose plus 200 p. p. m. of *dl*-isoleucine.

only one of the 23 amino acids capable of causing a specific response of this nature at this concentration.

Other amino acids can modify the action of *dl*-isoleucine on growth of tobacco seedlings (fig. 5). When 50 mg. per liter of *dl*-isoleucine was used with 20 mg. of another amino acid, a wide difference in response appeared. Increases in growth were effected by *l*-aspartic acid, *l*-cystine, glycine, *l*-histidine dihydrochloride, *l*-leucine, *d*-lysine dihydrochloride, *dl*-methionine, *dl*-norleucine, *dl*-β-phenylalanine, *l*-proline, and *dl*-threonine. The controls with *dl*-isoleucine had narrow leaves showing a diffuse chlorosis, whereas all admixtures except those with *dl*-alanine, *l*-hydroxyproline, and *l*-tryptophane produced seedlings with leaves having the clearly defined reticular chlorosis so characteristic of frenching. *dl*-Alanine, *l*-hydroxyproline, and *l*-tryptophane caused sharp decreases in yield. *dl*-Alanine and *l*-tyrosine, however, seemed to neutralize to some degree the abnormal morphological effects of *dl*-isoleucine, while *l*-hydroxyproline and *l*-tryptophane seemed to accentuate these (strap-leaf formation).

A summary of the characteristic symptoms of the various amino acid toxicities appears in table 4. The divergencies and similarities in gross morphology resulting from excesses of individual natural amino acids are listed. The quantities involved ranged from 0.25 to 2.5 mg. per seedling, if only those acids included in the table are considered. Symptoms of abnormality vary greatly and include loss of dominance, or death of the apical bud (retention of the rosette condition and inhibition of stem growth and of suckering); various leaf mottlings, streaks, and blotches; and narrow, strap, ruffled, twinned, and rim-bound leaves. Therefore, abnormalities in protein

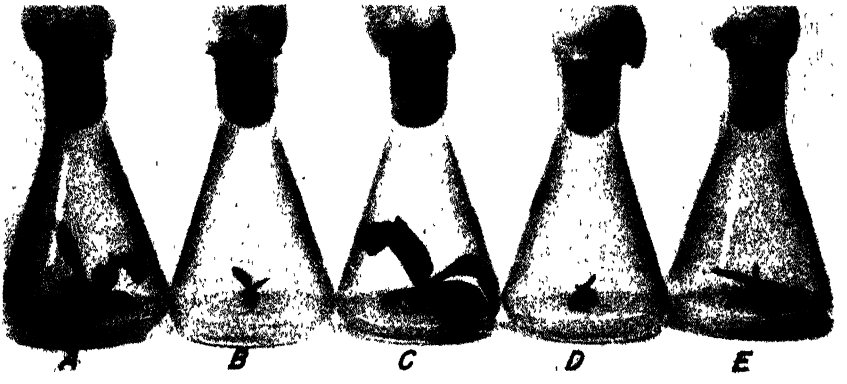

FIGURE 5.—Maryland Medium Broadleaf tobacco seedlings grown at 25° C. with 500 foot-candles of fluorescent white light and continuous illumination on mineral agar containing 2 percent of sucrose and 50 p. p. m. of *dl*-isoleucine plus additional amino acid at 20 p. p. m.: *A*, None (control); *B*, *dl*-alanine; *C*, glycine; *D*, *l*-hydroxyproline; *E*, *dl*-isoleucine.

metabolism consequent to disturbances of mineral nutrition or physical-environmental factors may comprise in part the mechanism through which such aberrations are made evident as abnormalities in gross morphology.

TABLE 4.—*Symptoms in tobacco seedlings, Maryland Medium Broadleaf, due to excessive concentrations of the more toxic natural amino acids*

Compound	Concentration	Symptoms of toxicity other than retarded growth
	P. p. m.	
dl-Alanine	5	Chlorotic spots at apex and margins.
l-Aspartic acid	50	Large, faintly chlorotic spots.
d-Glutamic acid	20	Large white blotches.
Glycine	40	Faint interveinal chlorosis.
l-Histidine dihydrochloride	50	Small, faintly chlorotic spots.
l-Hydroxyproline	5	Inhibition of buds; streak chlorosis, thick, down-curled leaves; brown, dead leaves; rim-bound leaves.
dl-Isoleucine	50	Loss of apical dominance, narrow leaves, mottling.
d-Lysine dihydrochloride	50	Reticular chlorosis.
dl-Norleucine	20	Inhibition of secondary root growth.
l-Proline	40	Faint interveinal chlorosis.
dl-Serine	40	General mottling.
dl-Threonine	50	White leaves.[1]
l-Tryptophane	20	Loss of apical dominance; isolated ruffled strap leaves, rim-bound, twinned, pointed, and withered-zone leaves; suckering.
dl-Valine	20	Large white blotches.

[1] White leaves are believed to be a general symptom due to excess quantities of amino acids; 20 p. p. m of the amino acids listed as causing this condition did not prevent normal morphological growth. Specific abnormalities in gross morphology will probably be found to occur at intermediate concentrations. An interesting symptom, root blackening, was also caused by high concentrations of tyrosine.

These growth effects of the free, natural amino acids, like those of D-mannose and commercial peptones (p. 89), would seem to minimize the need for assuming the existence of hormones regulating all the activities of plants, because it is evident that the ordinary metabolic products of the cell in themselves can exert a hormone action on the growth processes of plant tissues. The hormone action of other natural metabolites also may be the explanation for the similarities in response to dissimilar synthetic growth substances causing root initiation, epinasty, callus formation, and other growth correlations.

EFFECTS OF VITAMINS AND PEPTONES ON GROWTH

None of the vitamins used (table 5) appeared more than very slightly beneficial to the growth of Maryland Medium Broadleaf tobacco. Pyridoxine and ascorbic acid gave slight increases in yield which, however, did not exceed the limits of experimental precision.

It is interesting to note that certain peptones also brought about slight increases in yield. The important feature about these responses, however, was not the slight increases in yield that were obtained, but the evidence for the relative nontoxicity of peptones and proteoses as compared with amino acids and the peptide glutathione. Some of these peptones (Bacto-tryptose, Bacto-protone, Proteose-peptone) appeared favorable to retention of the rosette condition despite the short day, but the response did not appear regularly.

TABLE 5.—*Growth of tobacco seedlings, Maryland Medium Broadleaf, with 2 percent of sucrose plus vitamins or peptones in aseptic culture for 28 days at 25° C. and 500 foot-candles of fluorescent white light for 8 hours daily*

Control or compound (1.0 mg. per liter)	Average total yield	Appearance [1]	Control or compound (200 mg. per liter)	Average total yield	Appearance [1]
	Milligrams			*Milligrams*	
Control	19.6	S, 8	Casamino acids [2]	6 5	S, 2
Biotin [3]	20.4	S 8	Bacto-peptone [2]	22 7	S, 8
Thiamine chloride	14.8	S, 8	Peptone (Witte)	20 4	S, 8
Pyridoxine	23 8	S, 8	Malt extract [2]	18.8	S, 8
Nicotinic acid	19.5	S, 8	Yeast extract [2]	1 8	S, 2
Riboflavin [4]	19.0	S, 8	Bacto-tryptose [2]	22 8	R/2, 8
Ascorbic acid	22 4	S, 8	Bacto-protone [2]	18 0	R/2, 8
Calcium *d*-pantothenate	19.7	S, 8	Bacto-tryptone [2]	16 9	S, 8
p-Amino benzoic acid	19.8	S, 8	Proteose-peptone No. 4 [2]	23 1	R/2, 8
Folic acid [5]	19 3	S, 8	Neopeptone [2]	19.3	S, 8
Indole-3-propionic acid	11 7	S, 8			
Indole-3-acetic acid	11 1	S, 8			

[1] S, Shoot or stem; R/2, partial rosette. Rated from 0 (white) to 10 (dark green).
[2] From Difco Laboratories.
[3] 10 gammas per liter.
[4] 0.4 mg. per liter.
[5] 0.1 mg. per liter Courtesy of Prof. Roger J. Williams.

EFFECTS OF PURINES AND PYRIMIDINES ON GROWTH

Excesses of metabolites other than amino acids may behave similarly in producing abnormalities in gross morphology. At concentrations of 200 p. p. m., for example, adenylic acid, guanylic acid, uracil, guanine, guanosine, and hypoxanthine brought about faint mottling. Adenine caused formation of white leaves because of excessive toxicity. Faint, glossy, dark-green leaf blotches also were evident with xanthine, guanosine, and hypoxanthine. With all of these products of nucleic acid decomposition except adenine yields were almost normal. Growth with adenylic acid, uracil, and xanthine was, indeed, slightly better than that of the controls. Glutathione (table 2), a tripeptide, was also rather toxic and would probably cause production of specific symptoms at its toxic threshold.

DISCUSSION

Persistence of the rosette stage preliminary to shoot elongation varies considerably with different varieties of tobacco. The variety used in these experiments is characterized by a well-defined preliminary rosette stage. The day-length experiments of Allard and Garner (*1*) would indicate that duration of the rosette condition is briefer with long daily periods of solar illumination than with short ones. With the environmental conditions employed in these experiments this response appeared to be reversed. The plants grown with a short day began stem elongation almost immediately, whereas those in continuous illumination remained in the rosette stage for a month or more. The factor responsible was probably low light intensity, but high moisture and relative humidity may also have contributed to the reversal in response. Reversal is viewed primarily, however, as a low-nutrition response.

It is probably unnecessary to assume the presence of a rosette-forming hormone in this reaction of tobacco. The data seem to indicate that variations in the proportions and concentrations of ordinary biochemical constituents of the plant may bring about modifications in duration of the rosette stage. The difficulty in demonstrating this interpretation experimentally lies in proving that the components of the chemical mixture used to cause reversion to rosette formation are actually those so functioning in the plant. Biochemical data are needed.

The characteristic and specific response of tobacco seedlings to *dl*-isoleucine is of great interest, particularly if it proves to be the primary cause of frenching in tobacco.[3] This disease is of world-wide occurrence, but little is known of its cause. Response of tobacco to *dl*-isoleucine in aseptic culture is very similar to frenching in the field, considering the wide differences in these environments. Moreover, all 3 samples of this amino acid that were tried were equally effective and varied with the concentrations used. The ineffectiveness of about 60 other biochemical compounds to bring about a similar response would indicate the degree of specificity involved in this reaction. McMurtrey (*5*), however, was also able to duplicate the symptoms of frenching to some degree with thallium salts. Shear and Ussery (*8*) found that this tobacco disease occurred in the absence of thallium under field conditions.

The mechanism is unknown whereby specific characteristic symptoms in the green plant are brought about through mineral deficiencies and toxicities. Though an assumption of direct action might appear plausible for macronutrients, nutritive deficiencies of micronutrients at least could lead to characteristic syndromes only indirectly. The data presented on amino acids reveal the existence of a mechanism for the production of specific morphological abnormalities dependent upon irregularities in protein metabolism. Variations in formation and degradation of proteins under abnormal environments could lead to accumulation in excess of specific free amino and other acids,

[3] A similar response to *dl*-isoleucine is given by Xanthi Turkish tobacco in soil and solution culture in the greenhouse and by Connecticut Broadleaf in soil. Symptoms of mild frenching—bud inhibition, pale buds, narrow leaves with pale rims, and reticular chlorosis—were quite characteristic of the Connecticut Broadleaf variety in soil.

purines, pyrimidines, and peptides sufficient to produce a direct and harmful effect on growth and color of leaves, buds, and stems. The phenomena of amino acid toxicities have a range ample to account for at least some of the specific morphological symptoms encountered with disturbances in mineral nutrition. The validity of this interpretation is further supported by the data demonstrating the relative innocuousness of proteoses and peptones and by the modifications in symptoms resulting from amino acid admixture and from concentration.

Morphological abnormalities in plant growth are known to take place in darkness and are accompanied by an increase in free amino acids. Bennett (*2*) reported that iron chlorosis in spur leaves of pears is accompanied by an increase in soluble nitrogen.

SUMMARY

Seedlings of the Robinson strain of tobacco, Maryland Medium Broadleaf, were grown for 28 days in 200-cc. Pyrex Erlenmeyer flasks under aseptic conditions at 25° C. with 500 foot-candles of fluorescent white light. Each flask contained 50 cc. of a mineral-agar medium. Of about 60 sugars, amino acids, vitamins, and peptones added to the medium, only sucrose, D-glucose, D-fructose, and perhaps D-xylose were definitely beneficial to growth. Other compounds, particularly amino acids, were toxic at concentrations of 5 to 200 p. p. m. A specific toxicity response to *dl*-isoleucine duplicated most of the gross characteristics of the disease known as frenching, especially strap-leaf formation, reticular chlorosis, inhibition of all stem elongation, and large increase in leaf number. Admixtures of other amino acids with *dl*-isoleucine led to wide variations in response from diminution to accentuation of the symptoms of frenching. Individual acids caused distinctly different patterns of gross morphological abnormalities. It is suggested that excesses of free amino acids due to abnormalities in protein metabolism may contribute to the formation of characteristic patterns of symptoms in plants because of disturbances in mineral nutrition. Failure to pass through the rosette stage under short daily illuminations could be corrected partially by addition of D-mannose and certain peptones. Commercial peptones (peptones, proteoses) were relatively nontoxic as compared with the free amino acids.

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CONGENITAL MALFORMATIONS OF EYES OF SHEEP[1]

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INTRODUCTION

Maternal body temperature and moisture in mammals are sufficiently uniform to prevent these factors from interfering with normal development of the embryo. Lack of oxygen, excess of carbon dioxide, dietary deficiencies, and the presence of toxic substances, however, do interfere with and retard the rate of normal development.

The relation between a faulty maternal diet and anomalies in the offspring has been reported by many investigators. Hale (*5*)[2] and Moore, Huffman, and Duncan (*8*) found many congenital malformations in pigs and calves maintained on a vitamin-A-deficient diet. Warkany and his coworkers (*15*, *16*) described skeletal abnormalities in the offspring of rats due to maternal nutritional deficiencies. Franke and Tully (*4*) produced chick monsters by feeding grains which contained selenium, and Landauer (*6*) produced various types of abnormalities with organic selenium in creeper and normal strains of fowl. Beath et al. (*1*) reported malformations in the progeny of sheep which were grazed on seleniferous areas.

It is evident that selenium can interfere with the normal development of the embryo and that it can produce malformations in the progeny. The process by which selenium produces malformations was suggested by the investigations of Wright (*19*), when he demonstrated that selenium injures the oxidative mechanism of adult tissues. If selenium interferes with tissue respiration, those organs which have the highest growth rate during embryonic development, that is, the eyes, head, and extremities, would be the most seriously affected. This has been the case in all the malformations that the writers have observed in the lambs of sheep grazed on seleniferous areas.

Reports on selenium malformations up to the present time have dealt with the structural anomalies observed. The present paper presents observations on the gross and histological changes that occurred in the eyes of lambs from mothers which had grazed on seleniferous areas. The malformations involved the extremities and the eyes. This paper deals only with the gross and histological changes in the malformed eyes.

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[2] Italic numbers in parathenses refer to Literature Cited, p. 102.

MATERIAL AND METHODS

Two hundred and fifty malformed lambs were born in a group of 2,100 ewes grazed on a seleniferous area. Seventy-five percent of the malformed lambs died at birth and 10 percent died between the ages of 3 to 5 months. The lambs that lived longer were dwarfed, the extremities and eyes were deformed, and the reproductive organs were underdeveloped. Similar malformations were observed by Beath et al. (*1*) in animals on seleniferous areas in Wyoming.

The malformations of the eyes of 16 animals were studied. In order to observe the sequence of changes which occur during growth, the lambs were killed at ½, 1, 2, 3, 4, and 6 months of age. Analyses for selenium in the blood, liver, and kidney were carried out on all animals. In the younger group the selenium content of the liver ranged from 3.5 p. p. m. to 5 p. p. m. and in the older group from 0.8 to 1.5 p. p. m. In previous experiments the writers (*10*) had observed that about 30 percent of the daily selenium intake was eliminated in the urine provided no kidney injury was present. The tissues of newly born lambs, without congenital defects were free from or contained only traces of selenium.

All tissues were fixed in 10 percent formalin.

Studies of the various organs showed pathologic changes similar to those observed in the blind staggers type of selenium poisoning reported by Draize and Beath (*2*) and Rosenfeld and Beath (*12*).

GROSS CHANGES IN MALFORMED EYES

OBSERVATIONS

The eyes of 2- to 4-week-old lambs showed a single large transparent cyst protruding from the bulbar conjectiva. As the lambs matured these cysts ruptured and the eye proper could be observed. The normal position of the eye in the orbit was disturbed and the cornea was misplaced, either to the nasal or the temporal side. The cysts varied in size and were covered with fibrous tissue.

At necroscopy microphthalmia with multiple cysts and normal-size eyes without cornea and pupil were observed. The lenses were misplaced or absent and the whole organ appeared to be filled with clear, transparent fluid. The sclera was fused with the ethmoid, the frontal bones, or the floor of the orbit. In such cases the eyes were fixed and could not be rotated. Separation of the cysts from the orbit resulted in the rupture of the cysts (fig. 1, *A*). There were large pigmented epithelial and connective tissue folds overlapping the eyes (fig. 1, *B*). In the younger animals the eye appeared smaller (fig. 1, *C*) because of the smaller size of the cysts. In the older animals the only limiting factor imposed on the size of cysts was the space available within the orbit. The number of cysts varied from two to six per eye (fig. 1, *D*). The origin and insertions of the muscles of the eye could not be determined on account of the disorganized structural arrangement and the protrusion of the cysts. There were increased periorbital fat and some gelatinous embryonic tissue. The orbital cavity usually appeared normal, but occasionally some of the bones showed erosion and petechial hemorrhages caused by pressure of the cysts.

The eyes were sectioned through the anteroposterior axis. An eye of some sort was always present. No relation between the age of the

animals and the development of structures of the eyes could be observed. The increase in size was due to the enlargement of the cysts, not to development of the eyes. The malformations in the eyes of the same animal were not identical. Usually the deformities showed a more extreme manifestation in the right eye than in the left (fig. 2, A). Rawles ('9) observed that chick blastoderms of the head process on the left side have a higher developmental capacity than those on the right.

The various anomalies observed in the diffrent structurs of the eye may be described as follows:

Cornea.—The cornea was small and thickened and many times was

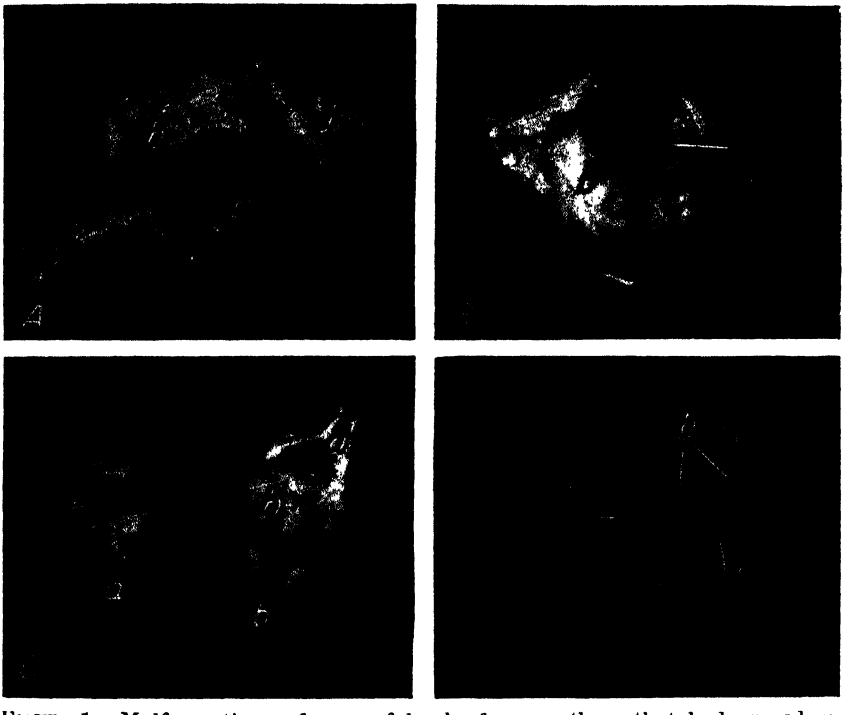

FIGURE 1.—Malformations of eyes of lambs from mothers that had grazed on seleniferous areas: *A*, *a*, Posterior view of eye, showing remnant of ruptured cyst which adhered to floor of orbit; *b*, posterior view of several large cysts. *B*, *a*, Pigmented epithelial fold covering anterior part of eye; *b*, large cyst with thin-walled elevated cysts; *C*, *a*, Eye of 2-week-old lamb showing microphthalmia; *b*, eye of 2-week-old lamb showing remnants of bulbar conjunctival cyst (*a'*) and posterior large cyst (*b'*). *D*, *a*, Misplaced eye; *b*, three small cysts; *c*, one large cyst. All X 0.7.

displaced laterally (fig. 2, *A*). A gray opacity obscured the normal division between the sclera and cornea. There was an extension of the episcleral tissue, and occasionally it showed some pigmentation.

Anterior and posterior chambers.—In many cases the division into anterior and posterior chambers was not well defined or was completely absent. In other cases the anterior chamber was shallow and the lens pushed forward, almost touching the cornea.

Lens.—Congenital absence of the lens was observed, usually when the eyes were rudimentary in size (fig. 2, *B*). Attachment of the lens to the retina or iris was observed in two cases. In one the lens appeared dumbbell-shaped and was covered by a continuous lens capsule. The lens was located postero-laterally; one part was within the eye while the other part projected into the cystic cavity (fig. 3, *A*).

Iris.—In all cases the iris was rudimentary or absent. The muscles of the iris were absent. Occasionally the rudimentary iris was attached and extended over the cornea.

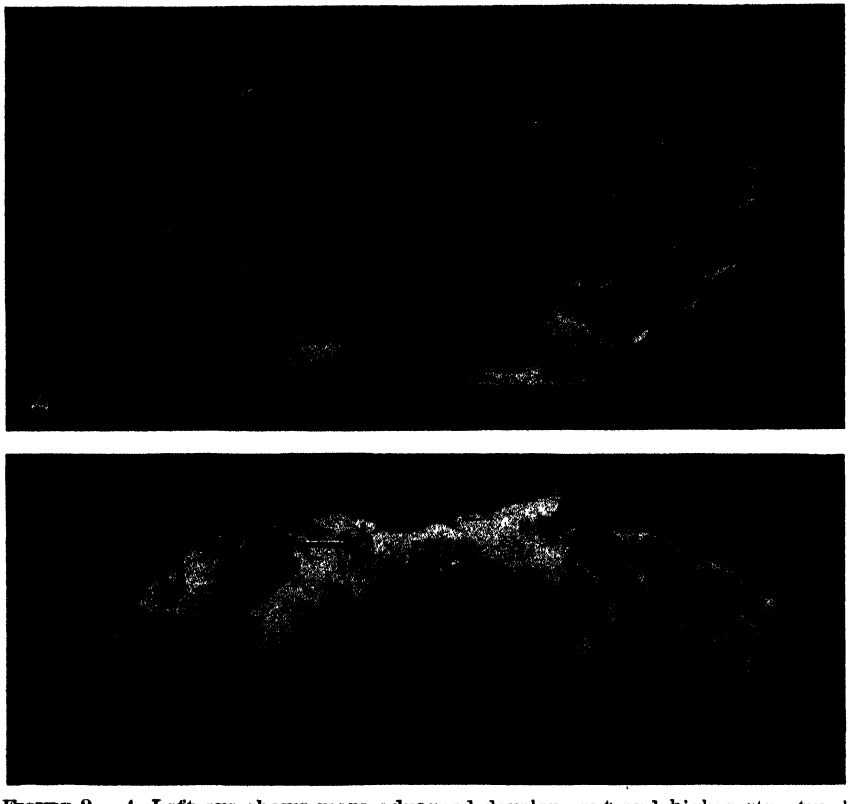

FIGURE 2.—*A*, Left eye shows more advanced development and higher structural differentiation than right eye: *a*, Cornea displaced laterally; *b*, cystic cavities and defects in walls of the eye; *c*, papillary projection on walls of cystic cavities. *B*, Microphthalmia rudimentary separation into anterior and posterior chambers; *a*, Eye filled with loose connective tissue, lens absent; *b*, displaced cornea; *c*, large cystic cavities with communicating sinuses. All × 0.7.

Optic nerve, retina, and choroid.—The optic nerve was absent, atrophied or calcified. Sometimes it ended in the sclera, and no direct connection between the eye and the optic nerve existed. In one case the optic nerve failed to develop, but an optic stalk and choroid fissure persisted. The optic stalk extended up to the rudimentary iris and remained embryonic in character, showing a developmental arrest (fig. 3, *B*).

The retina and choroid at the posterior part of the eye were normal in distribution. The retina extended over and covered the interior of the cysts.

Sclera.—The sclera around the primitive eye was thin, not well defined, and showed various defects which formed enlarged sinusus connecting with the cysts. The number of defects in the walls of the sclera depended upon the number of cysts present. Almost all the eyes showed some sort of scleral defect.

Cystic cavities.—The cystic cavities were filled with clear fluid which resembled vitreous humor. The cysts were bilocular or multilocular and the thin septum of the cysts had small defects which permitted intercommunication between the cystic cavities. The walls of the cysts were rough and uneven with various papillary projections. The walls of the cystic cavities were covered with epithelium which in some places appeared pigmented; in others pigmentation was absent.

The single cystic cavities in the older animals were 6 cm. in diameter, while the multilocular cavities were from 2 to 4 cm. in diameter. In

FIGURE 3.—*A*, Dumbbell-shaped lens that fills eye cavity: *a*, Lens protruding into cystic cavity. *B*, Eye showing persistence of optic stalk: *a*, Optic fissure, lens absent, indicating definite developmental arrest; *b*, large dilated cystic cavities; *c*, iris bent and fused to cornea. All × 0.7.

addition to the larger cysts, there were numerous smaller ones from 2 to 0.5 cm. in diameter, forming irregular and complex structural arrangements.

DISCUSSION

An understanding of the development of the various anomalies observed in the malformed eyes can be obtained only if we recognize the fact that embryonic growth and proliferation depend upon an unlimited supply of food and oxygen. Stockard (*13*) has pointed out that the normal development of the embyro depends upon the stability of certain factors in the environment. Embryonic death, as well as monstrous development, can be induced by changes in the conditions of moisture, temperature, oxygen supply, the action of various chemical substances, actinic rays, and dietary deficiencies. The malformations which are produced do not depend upon the specific teratogenic substance, but depend upon the time at which the developmental arrests occur. The development of a normal structure depends, not only upon a definitely located primordia, but also upon the time per-

mitted for its development. Therefore, it must attain its supremacy in growth and proliferation during a limited period. This time-limited opportunity for development is due to the growth competition between organs. Stockard (*13*) stated that if the entire embryo is depressed or has its developmental rate reduced, the rapidly developing structures will be affected more seriously than the slowly developing ones. When normal growth rate is resumed the slow-growing parts are able to regain their ordinary rates, but the rapid-growing organs are unable to resume their extraordinarily high rate of development, and, therefore, the rapidly developing organs lose their extraordinary advantages and the organ fails to develop or is malformed.

The abnormalities observed in these eyes shows developmental arrest, indicating that the anlage was there but the potential for growth and proliferation was lost. Some of the structures were in an embryonic state, which was well demonstrated by the presence of the optic stalk and the persistence of choroid fissue and embryonic lens. The different manifestations and the various grades of abnormalities indicated that injury did not occur in these animals at the same stage of embryonic development. Development was more advanced in some cases than in others, and consequently structural development was more advanced in some than in others when injury occurred. The postnatal age of the animals did not alter the defects in the structure of the eyes since the 3- and 6-months old animals had lens, iris, and corneal developments as primitive as those of a 2-week-old animal. Another interesting point observed was the fact that grossly the structures of the eyes did not show any of the degenerative changes that would be expected if the cysts exerted excessive pressure. The normal pressure in the various cavities was maintained through the sinuses and defects in the walls of the cysts and sclera. This pressure equilibrium suggests that the fluid was not foreign in origin, but was produced in the same manner as vitreous humor.

HISTOLOGICAL CHANGES IN MALFORMED EYES

OBSERVATIONS

Only a few histological studies of malformed eyes have been reported in the literature. Franke et al. (*3*) studied the effect of selenium in chick embryos but failed to give the histological or the gross changes that take place in the different organs. Warkany et al. (*14*, *17*) reported the histology of the malformations observed in the eyes of rats whose mothers were on a vitamin-A-free diet.

The eyes to be studied were fixed in 10 percent formalin, embedded in paraffin, and sections 10μ to 12μ thick were cut. The sections were stained with haematoxylin and eosin, with copper chrome haematoxylin, and with Mallory's connective tissue stain. From each eye 50 sections were taken at 100μ intervals.

Although the structural malformations of the eyes were varied and numerous, it was apparent that they were due to the failure of the various structures of the eye to fuse, lack of differentiation, lack of growth, or cellular proliferation. The failure of the sclera to fuse was the most frequent defect observed. When this happened the vitreous humor was permitted to flow into the mesoderm, where multiple cyst formation occurred.

The histological structure of the sclera indicated that there was not only an adherence of the sclera to the bony orbit, but also that endochrondral bone formation was present in the sclera. This process was not an abnormal calcification but an active bone formation. The sclera, in addition to the normal cellular structure, contained many undifferentiated mesenchymal cells.

The mesodern of the cornea failed to differentiate into its normal structures. The composition of the tissue was more like that of undif-

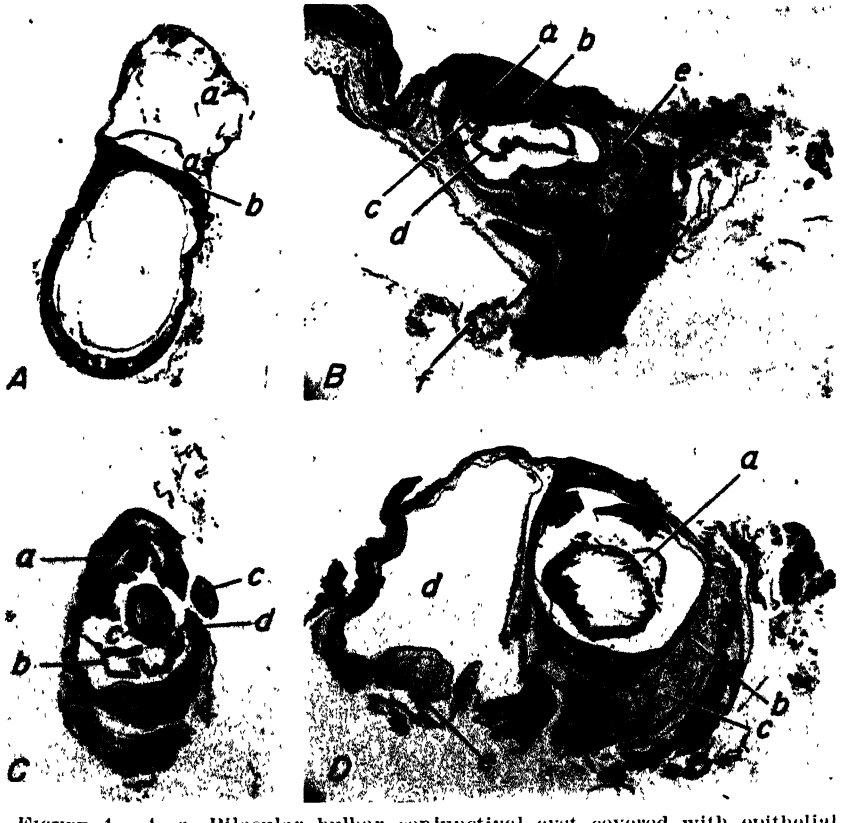

FIGURE 4.—*A*, *a*, Bilocular bulbar conjunctival cyst covered with epithelial cells; *b*, hyperplastic cornea. *B*, *a*, Pseudoanterior chamber; *b*, bent iris; *c*, ciliary processes extending over anterior part of eye; *d*, embryonic retina; *e*, embryonic tissue; *f*, papillary projection in cystic cavity. *C*, *a*, Eye shows microphthalmia with rudimentary anterior chamber; *b*, embryonic retina; *c*, lens extruded through defective sclera into cystic cavity, giving appearance of double lens because of curvature of lens; *d*, defect in sclera. *D*, *a*, Embryonic lens almost filling posterior chamber; *b*, thin sclera; *c*, embryonic tissue surrounding sclera; *d*, large lateral cyst; *e*, papillary projections on cyst shown in *d*.

ferentiated mesenchymal tissue with occasional pigmented cells and some connective tissue. The epithelium covering the cornea was normal in appearance except in a few cases in which epithelial hyperplasia was observed. In the eyes of the younger animals the bulbar conjunctiva formed systs which were covered with conjunctival epithelium (fig. 4, *A*).

The anterior chamber was rudimentary, shallow, or absent. The iris, at times, was absent, bent, or fused with the cornea. The bent iris formed a pseudoanterior chamber and brought about the displacement of the ciliary processes which extended over the enterior part of the eye or were attached to the cornea (fig. 4, *B*). The normal ciliary body was absent. Misplacement of the choroid occurred in one eye, and the choroid extended up to the anterior chamber where the vessels were dilated and a newly formed thrombus was present. The lens were rudimentary, misplaced, embryonic in character, or absent. Misplacement of the lens was due to its fusion with the iris or with the retina. The lens attached to the retina passed through the cystic cavity giving the appearance of a double lens (fig. 4, *C*). Occasionally the lens was highly cellular in appearance and embryonic in size and almost filled the cavity of the eye (fig. 4, *D*). The lens capsule was normal and the fibers were nucleated. The amorphous substance was unevenly distributed and there were many vacuoles.

In the abnormally everted portion of the cysts there was an absence of choriocapillary layers. Mann (*7*), discussing the abnormalities of the eye, states that the choriocapillaries of the choroid develop normally only when it is in contact with pigmented epithelium.

The retinal tissue exhibited various abnormalities. In many cases the retina revealed embryonic separation and was composed of two layers (fig. 4, *C*). The inner layer was much thicker than the outer and contained some neutral elements; the outer layer was composed of pigmented epithelium. In some cases this primary optic vesicle was converted into a cystic cavity. The retinal epithelium formed folds over the cystic cavities. At various areas in the cystic cavities there was a condensation of the epithelium, forming adenomatous arrangements (fig. 5, *A*). The core of the papillary projections was composed of nervous and connective tissue. There were areas in the walls of the cysts in which the retinal epithelium was folded and hyperplastic. The multilocular cysts were directly connected with the eye proper by multiple sinuses (fig. 5, *B*). The epithelium covering the sinuses was continuous with the cystic cavities and the retina of the eye. The walls of the cystic cavities were composed of fibrous tissue and lined with a perverse layer of retinal epithelium. Various interpretations as to the formation of perverse layering of the retina have been given. Wolff (*18*) suggested that this type of layering is an actual detachment of the retina resulting from the increased subretinal fluid which pushes the retina through the choroid fissure into the loose mesoderm. Cysts are formed in this manner by the condensation of the mesoderm outside the eye.

There were secondary cysts within the larger cysts which were covered both inside and outside with retinal epithelium. The secondary cysts formed near the optic nerve, in the retina, outside the sclera, and within the wall of the sclera or at the junction of the anterior and posterior chambers. Surrounding the cyst was a large amount of undifferentiated mesenchymal tissue. Within the cystic cavities occasionally there were structures which resembled ciliary processes containing pigmented epithelium. These were attached to the walls of the rudimentary eye and indicated that during the development of the eye some disorganization of the anlage occurred, as these structures developed outside the eye and within the cystic cavities.

The optic nerve frequently showed atrophy. In some of the eyes there was no connection between the optic nerve and the eye. In these eyes the nerve ended diffusely in the posterior part of the sclera. In two cases the optic stalk failed to develop into the optic nerve. There was also lack of differentiation in all other structures. The vitreous humor in the eye formed fine longitudinal fibrils and filled the cavity of the eye (fig. 5, *C*). The persistence of the optic stalk, the choroid fissure, and the embryonic vitreous indicated that developmental arrest occurred early in embryonic development. Mann (*7*), correlating the development of the eyes with the rest of the body, states that in the 35-mm. embryo the optic stalk is replaced by the optic nerve.

Within the eyes and surrounding the eyeball there was a great deal of undifferentiated tissue. The presence of this embryonic undifferentiated tissue indicated that development was inhibited and its

FIGURE 5.—*A*, Eye composed of two large cavities: *a*, Surrounded by sclera; *b*, surrounded by embryonic tissues; *c*, lens abnormal in shape and attached to retina; *d*, retina forming adenomatous arrangement at area of contact with lens; *e*, cyst outside eye proper lined with retinal epithelium; *f*, dilated cyst derived from retina. *B*, *a*, Defect in wall of sclera; *b*, intercommunicating sinuses lined with retinal epithelium; *c*, ciliary process attached near scleral defect at posterior part of rudimentary eye; *d*, dilated secondary cyst; *e*, retinal tissue forming adenomatous arrangement with dilated secondary cyst shown in *d*. *C*, *a*, Optic stalk; *b*, choroid fissure; *c*, filamentous vitreous; *d*, cornea, which appears homogeneous; *e*, single layer of epithelium covering cornea; *f*, muscle bundles disorganized and separated by large cyst; *fo*, muscle bundle.

potential for differentiation was lost. In contrast to this undifferentiated tissue were the tissues which maintained their developmental potential and differentiated at abnormal positions.

DISCUSSION

The malformed eyes investigated presented a bizarre histological picture. The malarrangements of these structures may be explained as a result of early embryological arrest and disturbance in the anlage. The factors which might have caused these changes are numerous. The developmental arrest may have been due to decreased oxygen caused by the action of selenium. The lack of growth and cellular proliferation at certain developmental stages may have been responsible for the rudimentary and malformed structural development. The decrease of vitamin A due to selenium may also have been a

contributing factor to these malformations. The writers (*11*) have observed a decrease in vitamin A and ascorbic acid in selenium poisoning, and Warkany (*14*) has described malformation of the eye with vitamin A deficiency where the vitreous was fibrous in character.

Normally ruminants can synthesize ascorbic acid, and the embryo is able to supply the necessary amount of ascorbic acid for its development. The decrease in ascorbic acid during embryonic development may have caused certain of the defects. The normal function of ascorbic acid is to maintain the integrity of the intercellular substance of mesenchymal supporting tissues. Only in one case were there definite signs of vascular damage suggesting avitaminosis, although the failure of the different mesenchymal structures to maintain their continuity during embryonic development may have been due to vitamin C deficiency.

The fact that the fluid present in the multiple cysts did not produce any gross or microscopic pathological changes may be explained by the fact that the intercommunicating sinuses maintained the normal pressure within the eyes.

The neural tissue within the cystic cavities, which formed the core of the numerous papillary projections, indicates that the epithelium of the retina served as internal covering for the cystic cavities.

The derivation of the endochondral bone from the sclera observed in some cases poses an interesting question. In the lower vertebrates the sclera is cartilagenous. Whether the presence of endochondral bone within the sclera in mammals indicates a reversion under stress to the less differentiated tissue or whether the bone formation was due to misplaced mesenchymal tissue cannot be stated definitely. However, the fact that endochondral bone was formed in the sclera indicates the pluripotentiality of mesenchymal tissue.

SUMMARY

A description of the gross malformations of the eyes observed in the progeny of ewes grazed on seleniferous areas is presented. Congenital microphthalmia, rudimentary eyes, displacement of the lens, absence of the lens, lack or normal division between the cornea and sclera, microcornea, and colobomas of the various structures are described.

Histological studies indicated that in the malformed eyes cellular and structural misplacement occurred during early embryonic development. The majority of the defects involved the structures which developed from mesenchyme. Endochondral bone formation within the sclera indicates the pluripotentiality of the mesenchymal tissue. Histological changes in the various structures of the eyes are discussed.

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REACTION OF MOUNTAIN BROME AND CANADA WILD-RYE STRAINS TO HEAD SMUT (USTILAGO BULLATA)[1]

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INTRODUCTION

In the Pacific Northwest and the Rocky Mountain States several species of forage grasses are commonly found affected with head smut (*Ustilago bullata* Berk.) in native stands and in plantings for hay or pasture, seed production, or soil and water conservation. Prominent among these species is mountain brome (*Bromus marginatus* Nees), a native perennial. This grass is one of the best forage species for use with sweetclover in the intermountain area. As part of a general program for improvement of the species, attention has been given to head smut resistance. This paper presents the reaction of several agronomically superior accessions of mountain brome to various races of head smut in controlled inoculation tests. *Elymus canadensis* L., another native species which is susceptible to the same races of head smut, has also been tested and the results are included herein.

MATERIALS AND METHODS

Fourteen accessions of mountain brome selected from field collections representing widely varying types from several Western States have been tested for degree of susceptibility to 4 races of head smut attacking this species. Two of these (race 5 and race 7) are among those described earlier,[2] and 2 (race 9 and race 12) are new races that have since been differentiated. The inoculations were performed by the partial-vacuum method. The inoculated seed was germinated in plant bands in the greenhouse in late winter, and after the seedlings were well above the soil they were moved outside for vernalization. The vernalized seedlings were transplanted to nursery rows in the spring, and most of them headed well the following summer. The same procedure was used for 12 accessions of *Elymus canadensis*.

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TABLE 1.—*Reaction of accessions of Bromus marginatus and Elymus canadensis to 12 collections of head smut from Bromus spp., and comparative virulence of the races*

Species inoculated and accession No.[1]	Smut (head-count basis)[2] caused by indicated race and collections from different species of *Bromus*												Collections infecting	Average smut	Susceptibility index
	Race 5			Race 7						Race 9		Race 12			
	B. marginatus		*B anomalus*	*B. catharticus*				*Bromus* sp.	*B inermis*	*B. marginatus*		*B. purgans*			
	M-C	M-W	M-L	M-I	M-I_1	M-I_2	M-R	M-T	M-Y	M-C_1	M-C_2	M-V			
Bromus marginatus:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Number*	*Percent*	
Wn-439	0	0	0	0 7	0 3	0	0	0	0	0	0	0	2	0.1	0.2
Wn-438	16.3	0	0	0	0	0	0	0	62.0	0	62 4	21 8	4	13 5	54
P-1841	94 6	95 4	97.9	63 1	47 4	67 0	70.7	4 0	49.2	90.1	83 5	92.9	12	71.3	856
P-2133	6.4	0	0	0	0	0	0	0	2 0	0	19 1	15.1	4	3.6	14
P-3972	83.1	58.6	49.2	28 9	38 2	42 4	44 1	16 0	52.2	100	88.0	86.9	12	57.3	688
P-3388	73 2	32.4	70 0	9 6	25 9	39 1	23 0	1 5	52 0	4.0	57 5	71.2	12	38.3	460
P-5355	87 3	0	0	0	3	0	0	0	61 1	84 6	40 1	57 5	6	27.6	166
P-5391	24 9	68 8	2.9	6 0	5 1	1 2	3 7	8 8	46 4	21.8	84 4	79.1	12	29 4	353
P-5722	87 0	91 1	1.4	75 6	67 5	65 4	65 3	19 4	93 0	85.3	64 5	89 5	12	67.1	805
P-6268	10.5	86 2	6 9	0	0	0	0	1 8	57 4	43 1	27.6	67.4	8	24.5	196
P-6328	12 2	5 9	97 7	46.1	36 5	7	0	1 3	2.4	27 6	10 7	76 1	11	26.4	290
P-7255	16.0	83 1	3	.4	4	0	0	3 8	1 2	3.4	2	73.4	10	15.2	152
P-4824	91 2	0	1 9	0	0	23 4	0	9	2.0	1.2	77.7	94.3	8	24 4	195
P-2725	43.3	93.8	81 6	0	0	0	0	0	0	100	65.7	13 2	6	33.1	199
Elymus canadensis:															
F-9	16 5	11 8	60 4	39 8	10 1	30 1	14 4	2 1	6 8	4	24.5	17 3	12	19 5	234
P-786	28.8	66 5	61 6	80 6	73.6	59 6	30 6	23 5	32.9	(3)	42.4	57 8	11	50 7	558
P-788	50 8	86 7	89.1	88 1	70 0	95 3	99 5	93 2	64 9	3.2	84 2	88.0	12	75 3	904
P-2389	71 1	35 4	68 2	93 6	97 6	99 4	84 6	85 5	59 9	21.3	88 4	82.9	12	73.2	878
P-2623	45.8	78 6	84 3	96 1	81 9	78.8	86 3	63 9	36 6	2.3	36.6	57 0	12	62 4	749
P-2624	42 9	74.8	81 9	93 1	42 3	66 9	81 1	64 3	26 1	3.5	34 2	47 3	12	54 9	659
P-3355	0	72 0	15 9	53 4	20 2	49 5	41 2	6 2	0	0	0	48 9	8	25.6	205
P-4597	36 3	30 1	75 7	86 9	86 6	77 6	83 2	64 6	35 4	0	13.3	63 4	11	54.4	598
P-4659	4 6	5 7	38.5	77.3	52.5	87 1	63 3	20 2	11 9	5.7	16.6	10 9	12	32.6	391
P-4669	0	54 9	15 9	4 8	6 4	0	6.6	24 2	2	0	5 2	23 0	9	11.5	104
P-4678	20.3	10 8	3 6	36 8	21 2	27 8	29 5	2.5	1 4	5 4	2 0	4.4	12	13.8	166
P-4681	0	47 6	0	30 8	26 8	27 0	17 7	10 2	31 8	28 9	1 4	1 2	10	18.6	186
Accessions smutted	22	21	21	20	21	18	17	21	23	19	24	25			
Average percent smut	37 0	45.8	38 7	38 9	31 2	36 1	32 0	19 9	29 7	25.3	40 0	51.4			
Virulence index	814	962	813	778	655	650	544	418	683	481	960	1,285			

[1] Wn numbers are those of the Washington Agricultural Experiment Station, P numbers, those of the Division of Nurseries, Soil Conservation Service, and the F number, that of the junior author.

[2] In most cases the number of heads per row ranged from 300 to 600 for *B. marginatus* and from 400 to 700 for *E. canadensis*.

[3] No stand.

RESULTS

In 1941, 14 accessions of mountain brome were each inoculated with 12 collections of head smut from widely separated localities in the Western States, representing 4 physiologic races. At the same time 12 accessions of Canada wild-rye were inoculated with the same smut collections. The purpose of the experiment was twofold: (1) To obtain a comparative evaluation of the resistance of the mountain brome and Canada wild-rye accessions previously determined as being agronomically superior and (2) to obtain a comparison of the virulence of the 4 races of head smut and of the collections which comprise these races. Data were taken in 1942 on a head-count basis, and the results are shown in table 1.

The data from these inoculations present some interesting results. In the first place, the differences between accessions in susceptibility to head smut are very great indeed. No 2 accessions of mountain brome gave the same reaction, the range of infection being 0 to 100 percent. Only 2 accessions appeared resistant to all 12 collections of smut; one (Wn–439) was practically immune, while the other (P–2133) appeared generally resistant or immune. Although several accessions of mountain brome were more or less infected by all 12 of the head smut collections, none of the accessions was definitely susceptible to all of them. The nearest approach to this was P–3972, for which the lowest percentage of smut was 16, obtained with collection M-T (race 7), and the highest was 100, obtained with collection M-C_1 (race 9).

A ready evaluation of the comparative resistance of the 14 accessions of mountain brome is provided in the susceptibility index in table 1. Neither the average percentage of smut nor the number of collections infecting is by itself a satisfactory index of susceptibility. The susceptibility index, the product of the average percentage of smut in a given accession and the number of collections infecting that accession, is considered a much better measure. By this measurement the outstanding immunity of Wn–439 is readily apparent as this accession has a susceptibility index of only 0.2. Other accessions also exhibit a high degree of resistance to most of the collections (Wn–438 and P–2133 with susceptibility indexes of 54 and 14, respectively), but they are more or less susceptible to 1 or 2 collections. Accession P–2133 (a reproduction of Wn–438) represents an early-maturing strain of mountain brome that has been in commercial production for about 10 years in Washington and probably elsewhere in the Northwest. Fortunately, it appears to be resistant.

Very much the same results were obtained with the 12 accessions of Canada wild-rye, except that the general susceptibility was even higher than with the brome accessions. The lowest susceptibility index was 104 and the highest was 904 (out of a theoretically possible 1,200). Accessions P–788 and P–2389 both were high in susceptibility.

Considerable differences in pathogenicity were observed in the 12 head smut collections used in the inoculations summarized in table 1. The disturbing fact came to light that all of the 12 collections seem to be different even though only 4 races are represented. Theoretically, it might be expected that all collections previously identified as belonging to 1 race should have given the same reaction on the 26

Bromus and *Elymus* accessions inoculated, but such certainly was not the case. The best instance for analysis is seen in collections M–I, $M–I_1$, $M–I_2$, M–R, M–T, and M–Y, all of which belong to race 7, on the basis of the reaction of the head smut differential grasses to these collections. However, on the basis of the reactions of P–1841, P–5355, P–6268, P–6328, P–2624, and P–4824 these 6 smut collections are all different. Similarly, the 3 collections belonging to race 5 and the 2 belonging to race 9 are easily differentiated on the basis of the reaction of some of the 26 host accessions used.

Further comparison of the 12 smut collections is possible by means of the virulence index shown at the bottom of table 1. The virulence index is the product of the average percentage of smut produced by a collection or race and the number of accessions of host plants which it was capable of infecting. Here, again, the collections within a race seem to differ. Thus, according to this index, collection $M–C_2$ is twice as virulent as collection $M–C_1$, and yet they are considered as belonging to the same physiologic race. However, it is not yet considered feasible to attempt to differentiate races by means of differences in virulence index. Rather it seems sufficient to recognize that the physiologic races previously described in *Ustilago bullata* are not genetic entities. The data in table 1 suggest that at least some of these races are composed of biotypes that can be separated by the use of additional hosts or strains within a host species.

The experiment just described yielded one result of a discrepant nature. Accession P–3368 was infected by all 12 collections, with an average of 38.3 percent of smut, and a susceptibility index of 460 (table 1). In previous preliminary tests this accession had exhibited a high degree of resistance and in the observational rows of the Soil Conservation Nursery at Pullman, Wash., it had remained smut-free for several years. The sudden susceptible reaction of P–3368 in the present experiment was, therefore, unaccountable. It was decided to conduct further inoculation experiments involving P–3368 and 5 other accessions of mountain brome of excellent agronomic qualities.

In the second experiment six accessions of *Bromus marginatus* were each inoculated with four races of head smut. In this test, inoculations were made with mixtures of collections of each race rather than with individual collections, because the primary interest was in testing the grass accessions for resistance. In the hope of finding in the susceptible accessions smut-resistant individuals that were agronomically desirable, the experiment was conducted and data were taken on a spaced-plant basis. The inoculated seed was planted in the greenhouse, and the seedlings were transplanted to individual, small, wood-veneer plant bands. A period of vernalization preceded the transplanting to the field in the spring of 1943. Most of the plants headed well in 1943, and reaction data were taken in 1944. These data are summarized in table 2.

The results of the second experiment (table 2) further indicate the apparent immunity of Wn–439 to head smut. This experiment reestablished the high degree of resistance in P–3368 and made the susceptible reaction of this accession in the first experiment even more subject to question. The high degree of susceptibility of P–3972 and P–5355 (susceptibility index 371 and 359, respectively) again is well

demonstrated. However, in no case were 100 percent of the plants infected with smut. The smut-free plants represent instances either of escape from infection or of genetic resistance. This investigation is being continued.

It will be noted that in this second experiment accession P–2133 appeared definitely susceptible to race 7 and race 12, a reaction quite in contrast to the general resistance manifest in the first test. In this connection it should be pointed out that preliminary inoculations in previous years had indicated that P–2133 is highly resistant to most collections of head smut but more or less susceptible to others.

The reactions of the six accessions of mountain brome shown in table 2 do not reveal any great differences in the general virulence of the four races of head smut. All have nearly the same virulence index.

TABLE 2.—*Reaction of 6 accessions of Bromus marginatus to 4 races of Ustilago bullata, and comparative virulence of the races*

Accession No.[1]	Race 5		Race 7		Race 9		Race 12		Races infecting	Average smut	Susceptibility index
	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected			
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	
Wn–439[2]	34	0	15	0	13	0	13	0	0	0	0
P–2133	101	3 0	73	37 0	84	17 9	94	53 2	1	27 8	111
P–3368	101	3 0	126	6 4	124	8	72	0	3	2 5	8
P–3972	97	99 0	92	84 8	79	93 7	54	88 9	4	92 8	377
P–5355	96	99 0	94	98 9	56	94 6	21	66 7	4	89 8	359
P–5391	111	50 5	129	24 8	104	63 5	123	98 4	4	59 3	237
Accessions smutted	5		5		5		4				
Average percent smut	42 4		42 0		45 9		51 2				
Virulence index	212		210		230		205				

[1] See footnote 1, table 1

[2] There was insufficient seed of this accession to provide the desired minimum of 100 plants per race of smut

The discrepant results obtained with accession P–3368 of mountain brome, as described, suggested error either in the identity of the seed used in the first experiment (table 1) or in the smut collections used as inoculum. The possibility of an error in the identity of the seed used seemed under the circumstances the more plausible, and it was decided to investigate that possibility first. Seed of P–3368 produced in 5 different years was used: 1936, 1938, 1940, 1941, and 1942. The 1940 seed was from the same lot that had been used in the first inoculation experiment, summarized in table 1, in which P–3368 was so generally susceptible. These five seed lots were each inoculated with six collections of head smut, five of which were the ones which produced the highest percentages of smut on P–3368 in the first experiment. The seed was inoculated and planted as in the other experiments. The 1936 seed was no longer viable, and no stands were obtained. The data taken on a head-count basis are presented in table 3.

TABLE 3.—*Comparative susceptibility of 4 seed lots of Bromus marginatus P-3368 produced in different years to various races and collections of Ustilago bullata*

Year seed was produced	Smut (head-count basis) produced by indicated race and collection						Average
	Race 5		Race 7 (M-Y)	Race 9 ($M-C_2$)	Race 12		
	M-C	M-L			M-V	M-Z	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1938	0	10.3	4.3	0	0	0	2.4
1940	35.4	40.3	62.5	74.1	84.2	60.3	59.5
1941	0	.5	.5	4.9	2.4	2.2	1.8
1942	0	0	0	0	.9	0	.2

A comparison of the results with the four seed lots of P-3368 in table 3 indicates that the 1940 lot, which gave the discrepant results in the first experiment, was not accession P-3368, as labeled, but probably represents some susceptible selection of *Bromus marginatus* or perhaps another accession of the same grass.

DISCUSSION

From the foregoing results it appears that within the species *Bromus marginatus* there are strains varying markedly in their reaction to races or collections of head smut. Two types of mountain brome of economic importance are easily distinguished by their growth habits. The first, represented by Wn–438 and P–2133, is an early-maturing, fairly leafy strain and the second, represented by Wn–439 and P–3368, is a late-maturing, very leafy strain. Both types are satisfactory with regard to seed yield, but the latter type by virtue of its lateness and leafiness is the more desirable strain for use with sweetclover.

Before accessions or selections are released for increase or commercial production, reaction to head smut should be determined; and since resistant strains are available, preference should be given to these. While head smut can be controlled by proper seed treatment, the use of resistant or immune strains eliminates the necessity of this operation. In the present investigations, two accessions of mountain brome were outstanding for their resistance to head smut: Wn-439 and P-3368. The first is a Washington Agricultural Experiment Station selection; the second is an increase of Wn–439 made by the Soil Conservation Service at Pullman, Wash. Further study of strains of mountain brome is being made with the intention of releasing the one that proves most resistant to smut and at the same time is superior for use as forage.

SUMMARY

Fourteen accessions of mountain brome (*Bromus marginatus*) and 12 of Canada wild-rye (*Elymus canadensis*) have been tested for susceptibility to races of head smut (*Ustilago bullata*) known to attack mountain brome. Considerable differences in degree of susceptibility were found in the mountain brome accessions, ranging from high resistance to high susceptibility. The same was true for Canada wild-rye, but this species was more generally susceptible than mountain brome.

Since some lines of mountain brome are highly susceptible to head smut, it is essential that the reaction to this smut be determined before strains of the grass are increased for seed or released for commercial production.

It appears probable that none of the physiologic races of *Ustilago bullata* that have been differentiated and described represents a genotypic entity, but rather that they are phenotypes whose component parts may be separated by the use of additional differential hosts or strains within a host species.

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VARIABILITY IN PHOMA LINGAM[1]

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INTRODUCTION

In the Puget Sound section of Washington a species of *Phoma* has been found to occur regularly as a minor leaf spot and stalk rot pathogen of seed plantings of cabbage (*Brassica oleracea* var. *capitata* L.) and Chinese cabbage (*B. pekinensis* (Lour.) Rupr.). On the latter host losses resulting from such infections have been important. The same fungus has also been found to cause mild infection on seed plants of turnip (*B. rapa* L.) and rutabaga (*B. napobrassica* (L.) Mill.).

The Puget Sound section has become this Nation's chief producer of cabbage seed primarily because seed grown there does not carry *Phoma lingam* Fr. (Desm.), the organism that causes blackleg, and *Xanthomonas campestris* (Pam.) Dows., the one that causes black rot. It became of interest, therefore, to determine the pathogenic capacity of these isolates of *Phoma* and to determine their relation both to the cabbage blackleg organism and to the organism of dry rot of rutabaga in Europe (*8*)[2] and New Zealand (*2*). This paper presents a description of the *Phoma* that occurs in the Puget Sound section and considers its variability in relation to that of *P. lingam* found associated with typical blackleg of cabbage in other sections of the United States.

REVIEW OF LITERATURE

In 1791 Tode (*9, p. 51 and pl. XVI, fig. 126*) described the blackleg organism of cabbage and named it *Sphaeria lingam*. Because he found the fungus occurring on dead cabbage stems, he considered it a saprophyte. In 1849 Desmazières (*3*), however, collected the same fungus on living plants and transferred it to the genus *Phoma*. In Denmark in 1894 Rostrup (*8*) described a disease of rutabaga that he attributed to an organism which he named *P. napobrassicae*. In 1918 Henderson (*5*) gave a detailed account of the occurrence of *P.*

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[2] Italic numbers in parentheses refer to Literature Cited, p. 132.

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lingam in Wisconsin, where it caused severe losses in cabbage. Henderson did not mention variability of the causal organism, but he described two kinds of pycnidia that it produced. He stated that considerable evidence existed for placing *P. napobrassicae* in the species *P. lingam*.

In 1905 Kirk (*7*) observed a dry rot of rutabaga in New Zealand and identified the causal organism as *Phoma napobrassicae* Rostrup. Cunningham (*2*) studied the growth rate, type, and pathogenicity of about 400 isolates from seed and other plant parts of rutabaga affected with dry rot. On the basis of growth rate and pathogenicity to nongrowing rutabaga roots he divided the isolates into 2 groups. Group 1 contained fast-growing, nonstaling forms which were weakly pathogenic; group 2 contained slow-growing, staling forms which were strongly pathogenic. Cunningham also studied cultures of *Phoma* obtained from cruciferous plants in Wisconsin, the Netherlands, and Africa and concluded that the dry rot in New Zealand, England, and continental Europe was caused by the same organism as blackleg of cabbage in the United States. He suggested *P. lingam* (Tode) Desm. as the correct binomial for the pathogen.

Cunningham's study of seed transmission of dry rot stimulated a reinvestigation of the disease in England and Ireland, since most of the seed planted in New Zealand was imported from those countries. In 1933 Hughes (*6*) essentially confirmed Cunningham's findings of strain-group relations as far as growth rate was concerned, but he was not able to confirm his separations on the basis of pathogenicity. He suggested the existence of one strain of *Phoma lingam* that occurred primarily on rutabaga in Europe and New Zealand and of another that was associated with cabbage and various other crucifers, but not with rutabaga. Hughes presented limited evidence to support this idea. He concluded further that the strain associated with rutabaga was identical with the American blackleg organism and that both belonged to the species *P. lingam*.

In 1934 Buddin (*1*) reported the results of his reinvestigation of dry rot in England. He found that five of the seven representative isolates sent him by Cunningham did not conform to *Phoma lingam* and were weakly pathogenic. In fact, he identified some of them as belonging to other genera of fungi. He was able to show, however, by single-spore cultures that considerable variability existed within *P. lingam*, largely in rates of growth and amounts of staling. He interpreted his observations to mean that the isolates comprised a graded series rather than clear-cut groups.

Grove (*4, v. 1, p. 70*) reduced Rostrup's species to varietal status as *Phoma lingam napobrassicae* (Rostr.) Grove.

From this discussion it is evident that, according to the International Rules of Botanical Nomenclature, the cabbage blackleg organism would be cited as *Phoma lingam* (Fr.) Desm., since Tode is pre-Friesian.

MATERIALS AND METHODS

The sources of all cultures studied are given in table 1.

Single-spore cultures were obtained by pouring a very dilute conidial suspension from the mass cultures over the surface of water agar. After incubation for 24 to 36 hours the individual germinating spores

were lifted with a needle under a stereoscopic microscope and each was planted in a culture tube containing suitable medium. The following system of culture designations was used: A letter, a group of letters, or a word followed only by a number represented a monoconidial isolate from one of the original mass cultures listed in table 1 (for example, W11 and PS40). Sectors arising from such single-spore cultures were indicated by placing after the designation of the single-spore culture the letter M and a number to indicate the order in which the sector arose. Thus, W11M1 was the first mycelial sector from W11. Culture W11M2M1 was the first mycelial sector of the second mycelial sector from W11. The letters SS and a number following a culture designation signified single-spore transfers of the isolate in question (for example, S26SS1).

All spore measurements were made under an oil-immersion lens at 900 magnifications. The spores were taken from a heavily sporulating culture on potato-dextrose agar and were mounted in glycerol jelly.

All isolates that were studied comparatively were grown on media from the same source.

TABLE 1.—*Sources of all Phoma isolates studied*

Culture or disease designation	State of origin	Contributor	Host	Key to single-spore isolates
Phoma lingam	Wisconsin	J. C. Walker	Cabbage	W1 to W200.
Cabbage blackleg	Iowa	W. J. Hooker	do	Iowa-1 to Iowa-30.
Do	New York	C. Chupp	do	NY1 to NY28
Do	Wisconsin	R. H. Larson	do	W300 to W550
Phoma sp.	Oregon	I. H. Vogel	do	S1 to S50
Do	Washington	G. S. Pound	Chinese cabbage	PS1 to PS150.
Do	do	do	Cabbage	PS200.
Do	California	K. Baker	Sweet alyssum	C1 to C25.

EXPERIMENTAL RESULTS

ISOLATION OF STRAINS

From a mass culture of *Phoma* from a Chinese cabbage plant in the Puget Sound section, 150 monoconidial isolates were established on potato-dextrose agar. As no variability was noted among these isolates, one, PS40, was selected for further study. A second monoconidial culture, PS200, obtained from a mass culture from a cabbage plant infected by *Phoma* in the Puget Sound section was also studied in some detail since it differed slightly from PS40. A third culture, C1, of this group, which differed only slightly from both PS40 and PS200, was sent to the writer from California by Kenneth Baker. It was originally isolated from a seed field of *Lobularia maritima* (L.) Desv.

Two hundred monoconidial isolates were established from a culture of *Phoma lingam* received from J. C. Walker, of the University of Wisconsin. These were designated as W1 to W200. From these isolates 2 distinct culture types were obtained on potato-dextrose agar. Six (represented herein by W43) of the 200 isolates were fast-growing cultures which produced an abundance of aerial mycelium and relatively few pycnidia. The remaining cultures (represented herein by W11) were all very slow in growth rate and produced an abundance of pycnidia, but very little aerial mycelium.

In February 1944 I. H. Vogel, of the Associated Seed Growers, Inc., gave the writer a leaf with a single *Phoma* lesion, suspected to be blackleg, from a cabbage plant growing at Salem, Oreg. Fifty monoconidial isolates (designated S1 to S50) were made from the original culture obtained from the lesion. Three distinct culture types were found among these monoconidial isolates. One type (represented by S1) was very similar to the slow-growing type obtained from the Wisconsin culture. A second type (represented by S39) differed from the S1 type only in the production of small, black, sclerotiumlike bodies on the potato-dextrose agar. The third type (represented by S26) was a fast-growing culture, which differed from the fast-growing isolates of the Wisconsin culture in that it produced fewer pycnidia and a greenish-black pigment in the mycelium.

Cultures obtained from blackleg material supplied from Iowa and New York were alike and practically identical with the fast-growing isolates obtained from the Wisconsin culture. Monoconidial isolates Iowa–11 and NY1 were selected to represent the cultures from Iowa and New York, respectively. From cabbage blackleg material received from Wisconsin in 1945, 250 single-spore isolates were established. All of these isolates (W300 to W550) were essentially alike and practically identical with the cultures from New York and Iowa and with the fast-growing isolates obtained from the previous culture from Wisconsin. Thus, the following arbitrary groups and subgroups of *Phoma*, according to their characteristics on potato-dextrose agar, were obtained from cultures isolated from cruciferous plants.

Group 1:
- A. Slow-growing isolates of Wisconsin culture. (W11.)
- B. Slow-growing isolates of Oregon culture that produced no sclerotiumlike bodies. (S1.)
- C. Slow-growing isolates of Oregon culture that produced sclerotiumlike bodies. (S39.)

Group 2:
- A. Fast-growing isolates of Wisconsin cultures, as well as cultures from Iowa and New York. (W43, NY1, Iowa-11, and W307.)
- B. Fast-growing isolates of Oregon culture. (S26.)

Group 3: Puget Sound isolates and the isolate from California. (PS40, PS200, and C1.)

CULTURAL CHARACTERS OF STRAINS

Growth Type

Examination of table 2 will reveal that the various representative isolates had distinct cultural characters. Isolates PS40, PS200, and C1 were distinct from all others in the production of a yellow to brown, water-soluble pigment in the medium. Mycelial growth was much coarser than that of other isolates, and a yellow to tannish-brown pigmentation of mycelium was very distinct. Sporulation was moderate. The pycnidia of PS40 and C1 usually occurred in clusters, but occasionally developed singly.

Isolates W43, NY1, Iowa–11, and W307 showed only minor differences in type of growth. All produced an abundance of white to gray or grayish-brown aerial mycelium which ultimately became grayish black. No staling occurred at room temperature. Sporulation was slight and pycnidia developed singly.

Isolate S26 was similar in many respects to NY1 and Iowa–11, but it was distinct on potato-dextrose agar in the production of a mycelium that had a powdery sheen and later developed a characteristic greenish-black pigmentation from the center of the colony out. Sporulation was very slight; pycnidia developed singly and were irregularly scattered over the agar surface.

TABLE 2.—*Characteristics of representative Phoma isolates grown on malt-extract, oatmeal, and potato-dextrose agars for 6 weeks at room temperature*

Isolate	Malt-extract agar	Oatmeal agar	Potato-dextrose agar
W11	Growth slow; staling ultimately complete, mat outline very irregular with dendritic pattern during first 2 weeks, becoming rather regular later, but always less circular than those of other isolates. Aerial mycelium fairly abundant, white at periphery of mat, greenish black at center. Pycnidia very abundant, small, black. Black pigment beneath mycelial mat with age.	Growth moderately fast; staling slight; mat outline regular. Aerial mycelium scant (much less than on malt-extract agar), grayish white, in somewhat concentric rings, giving a zonate appearance. Pycnidia very abundant, scattered evenly over surface, small, black. Slight black pigment beneath mycelial mat with age.	Growth fairly slow, staling moderate, mat outline regular to very irregular, often with dendritic pattern. Aerial mycelium scant, grayish white, submerged mycelium milky white. Pycnidia very abundant, scattered evenly over surface and submerged, small, black. Slight black pigment beneath mycelial mat with age.
S1	Growth slow, staling ultimately complete; mat outline always very regular. Aerial mycelium scant but more than on other agars. Pycnidia very abundant, scattered evenly over surface, small, black. Zonation distinct with dark center, grayish-brown midzone, and white periphery. Differing from W11 chiefly in conspicuous zonation and regularity of mat outline.	Growth moderately fast, staling slight, mat outline very regular. Aerial mycelium very scant. Pycnidia very abundant, scattered evenly over surface at first, but in concentric rings later, small, black.	Growth moderately fast at first, staling complete after 3 weeks (ultimately distinctly more than that of W11), mat outline very regular. Aerial mycelium very scant. Pycnidia abundant, scattered evenly over surface and submerged, small, black. Slight zonation of alternating light and dark areas very pronounced, black pigment beneath mycelial mat very different from that of W11 with age.
S39	Almost same as S1 except for more conspicuous zonation.	Growth moderately fast; staling slight, mat outline regular. Aerial mycelium scant. Pycnidia very abundant, very conspicuously, concentrically zonate (very different from W11 and S1). Resembling S1 in other respects.	Growth moderately fast at first, staling complete with age; mat outline very feathery. Pycnidia scant. Sclerotiumlike bodies scattered over surface, but piling up in center, about 0.5 mm in diameter, black. Differing from growth of S39 on other agars and differing distinctly from all other isolates in production of sclerotiumlike bodies. Pigment beneath mycelial mat very pronounced, black with age.
S26	Growth moderately fast (much slower than on potato-dextrose agar); staling very little, mat outline very regular. Aerial mycelium abundant, cottony, elevated in center, later becoming dirty white, tending somewhat to collapse and form concentric rings. Pycnidia scant, covered by mycelium. Black pigment developing beneath fungus mat from center out.	Growth very fast; staling none, mat outline regular. Aerial mycelium moderately abundant, white at first, but becoming yellowish white, dense in center producing a bull's-eye effect. Pycnidia very scarce. Slight pinkish pigment developing in center.	Growth very fast; staling very little, mat outline very regular. Aerial mycelium moderately abundant, coarse, powdery white at first, becoming greenish black from center out until whole mat involved. Pycnidia scant, relatively large. Differing from other isolates in greenish-black pigment and powdery sheen of mycelium.

TABLE 2.—*Characteristics of representative Phoma isolates grown on malt-extract, oatmeal, and potato-dextrose agars for 6 weeks at room temperature*—Con.

Isolate	Malt-extract agar	Oatmeal agar	Potato-dextrose agar
Iowa-11	Growth moderately fast; staling none; mat outline very regular. Aerial mycelium abundant, elevated in center, white. Pycnidia scant. Black pigment developing beneath mycelial mat. Resembling S26.	Growth very fast; staling none, mat outline regular. Aerial mycelium moderately abundant (much less than on malt-extract agar). Pycnidia scant. Pigment developing slowly beneath mycelial mat, black.	Growth very fast; staling none; mat outline very regular. Aerial mycelium moderately abundant, white to gray. Pycnidia at center scant, large; pycnidia later scattered throughout, small. Black pigment developing beneath mycelial mat; agar occasionally pinkish. Differing from W11 and S1 in type of growth and amount of sporulation; resembling S26 in many respects.
NY1	Same as Iowa-11 except for much flatter mat center.	Same as Iowa-11 except for less abundant mycelial growth and greater zonation with alternate rings of gray and tan-white mycelium	Same as Iowa-11 except for being zonate at the periphery of mat.
W43	Almost same as Iowa-11; resembling NY1 except for elevated center like that of Iowa-11.	Almost same as Iowa-11.	Almost same as Iowa-11 and NY1 except for dense greenish-black pigmentation of central zone, which becomes studded with pycnidia more quickly than Iowa-11 and NY1.
W307	Not studied.	Not studied.	Very similar to Iowa-11, differing in that mycelium is grayish brown and sometimes sulfur yellow in central zone.
PS40	Growth moderately fast; staling none, mat outline very regular. Aerial mycelium very abundant, very coarse, white at first, but rapidly turning yellow to tan to brown from center out and remaining much darker at center. Pycnidia scant to moderately abundant. Yellow to brown pigment diffused throughout agar.	Growth very fast; staling none; mat outline very regular. Aerial mycelium very abundant, coarse, yellow brown. Pycnidia moderately abundant, usually in clusters rather than evenly scattered over surface. Conspicuous pigment developing throughout, pink at first, becoming yellowish brown.	Growth very fast; staling none; mat outline regular. Aerial mycelium abundant, very coarse, white at first, but changing to yellow to yellowish brown, becoming much darker in center and producing a bull's-eye effect. Pycnidia like those developed on oatmeal agar; also strictly aerial pycnidia in old cultures, abundant, black. Yellow pigment diffused throughout agar.
PS200	Same as PS40 except for noticeably lighter center of mat and finer mycelium.	Not studied.	Same as PS40 except for aerial mycelium, white at first and becoming less brown and more zonate, and pycnidia more abundant and in concentric zones.
C1	Not studied.	----do----	Very similar to PS200; cottony white mycelium, becoming more grayish brown than tannish brown. Pycnidia clustered in conspicuous zonate rings.

Isolate W11 was distinct from all others, but it was closest to S1. On potato-dextrose agar it produced very little aerial mycelium, but rather a milky-white submerged growth. Its growth outline was mostly irregular and often assumed a markedly dendritic pattern. Sporulation was much more profuse than that of the fast-growing cultures. Pycnidia were closely arranged with no semblance of concentric zonation. Ultimately cultures became staled and dark. The growth outline of isolate S1 was regular in contrast to the dendritic pattern often produced by W11, and a greater tendency for zonation

existed; ultimately staling was more pronounced and cultures assumed a distinctly blacker appearance.

Isolate S39 differed from S1 on potato-dextrose agar in the production of numerous black sclerotiumlike bodies (about 0.5 mm. in diameter) instead of small pycnidia. Pycnidia later developed in these sclerotiumlike bodies, and spore masses were discharged. Spore production, however, was much less than that of S1. A few small pycnidia like those of S1 were occasionally produced along with the sclerotiumlike bodies. On malt-extract and oatmeal agars, however, these sclerotiumlike bodies were not produced; instead, a profuse development of small, black pycnidia occurred and the growth of isolate S39 was practically identical with that of S1.

The cultural characteristics of representative isolates at room temperature are shown in figure 1.

Growth type at 30° C. was different from that at room temperature for most of the isolates studied. Isolate PS40 produced a much more intense pigmentation of agar, the pigment ultimately becoming plum red. The mycelium was coarser and much darker than at room temperature. Isolates Iowa–11 and NY1 produced white to grayish-white mycelial mats with less mycelium and almost none of the pigmentation found at room temperature. Isolate S26 produced an elevated mycelial mat with considerable grayish-black aerial mycelium and a hard stromalike growth beneath the mat at 30°. Both W11 and S1 were regular in outline at 30° and produced a raised growth with considerable aerial mycelium, gray in W11 and grayish black in S1. A hard stromalike growth developed beneath the mat, and pycnidia were fewer than at room temperature.

Growth Rate

Tables 3 and 4 show that isolates W11, S1, and S39 were very similar in growth rate at room temperature but that minor differences occurred. Staling of S1 was more pronounced and its ultimate growth was less than that of W11. Regardless of the type of medium used W11, S1, and S39 always grew slowly and eventually staled. Frequently fan-shaped growth developed from portions of a staled culture, giving it a very irregular outline. These areas of new growth were often numerous enough to coalesce and the result was a considerable increase in growth diameter. Isolates S26, W43, NY1, Iowa–11, and PS40 were all very similar in growth rate, and staling rarely occurred at room temperature. The growth outline was always regular, regardless of type of medium used. Isolate W43 showed a considerably slower growth on rutabaga-dextrose agar than did others of this group. Isolates C1 and W307 were not included in these comparative tests.

In table 5 comparative growth rates at room temperature and 30° C. for some of the isolates are given. At 30° isolates W11 and S1 were very similar, both being much more severely staled than at room temperature. S26, a fast grower at room temperature, was severely staled at 30° and ultimately its growth was no greater than that of S1. Isolates NY1 and Iowa–11 showed no appreciable difference in growth at the two temperatures until after 8 to 10 days, when growth at the high temperature became less than at room temper-

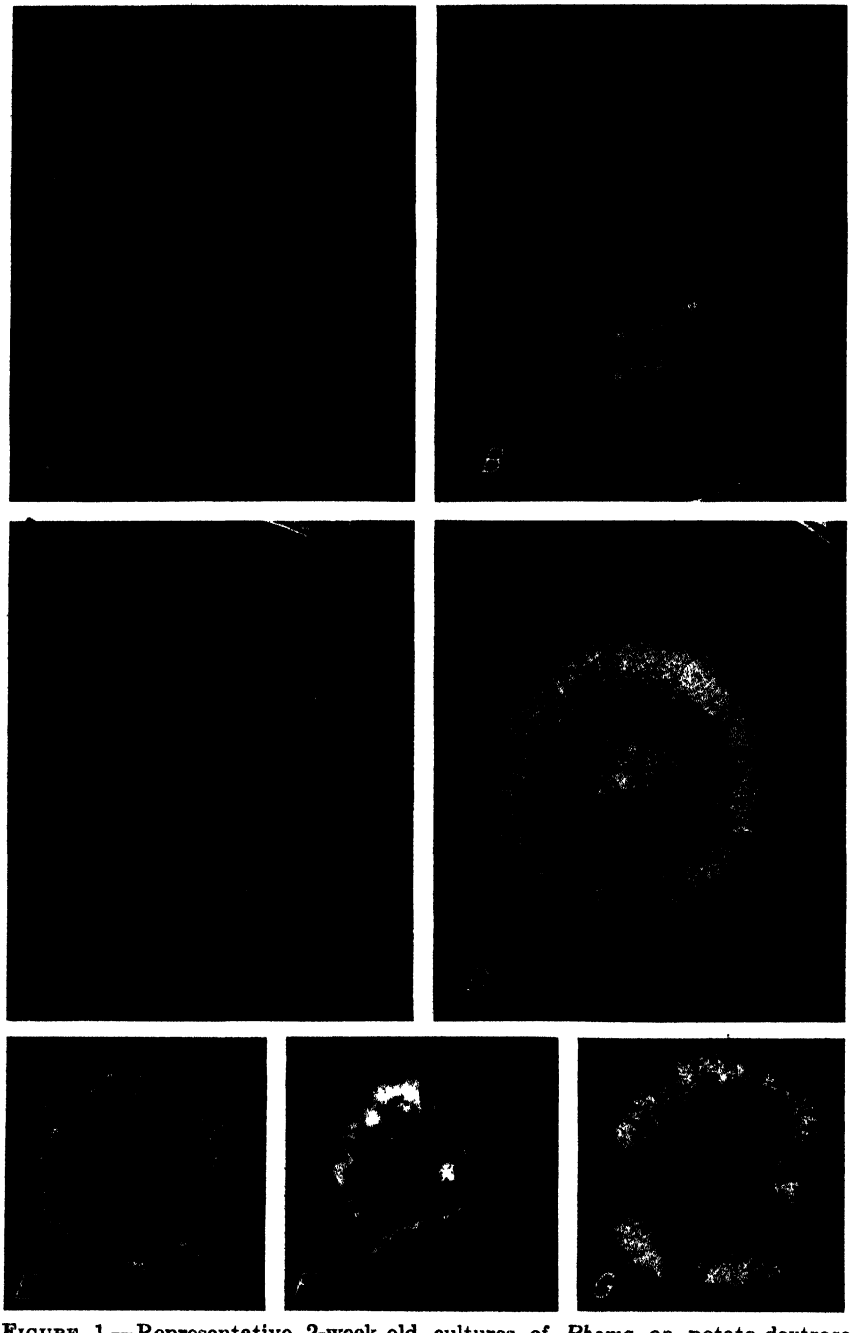

FIGURE 1.—Representative 2-week-old cultures of *Phoma* on potato-dextrose agar at room temperature: *A*, Iowa–11; *B*, PS40; *C*, W43; *D*, S26; *E*, S1; *F*, W11; *G*, S39.

ature. Isolate NY1 was staled more than Iowa–11; it was ultimately completely staled. Growth of both isolates at 30° fell considerably short of that at room temperature. Isolate PS40 had approximately the same growth rates at the two temperatures.

TABLE 3.—*Growth rates of representative Phoma isolates in 6-ounce bottles, at room temperature, on malt-extract and potato-dextrose agars*

[Each value is an average from 3 single-spore cultures]

Medium and isolate	Growth in indicated period (days)							
	4	7	11	13	15	18	20	40
	Millimeters	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*
Malt-extract agar:								
W11	2	7	10	12	15	22	26	73
S1	2	7	12	14	18	26	29	71
S39	2	7	12	14	19	25	30	69
S26	3	8	18	24	31	37	43	101
W43	4	11	20	27	39	48	55	(1)
NY1	4	12	24	37	47	57	65	(1)
Iowa–11	4	14	26	38	47	57	64	(1)
PS40	5	18	27	37	49	56	63	(1)
Potato-dextrose agar:								
W11	2	9	30	40	52	59	66	92
S1	2	14	32	38	45	51	53	60
S39	2	11	32	39	49	56	63	76
S26	6	28	50	65	75	87	93	(1)
W43	8	28	48	61	81	88	(1)	(1)
NY1	8	33	58	74	88	(1)	(1)	(1)
Iowa–11	8	32	58	73	88	(1)	(1)	(1)
PS40	11	35	57	73	84	(1)	(1)	(1)

[1] Growth had filled bottle and was beyond measurement.

TABLE 4.—*Growth rates of representative Phoma isolates in 6-ounce bottles, at room temperature, on rutabaga-dextrose agar*

[Each value is an average from 3 cultures inoculated with 3-mm. agar disks from 10-day-old cultures]

Isolate	Growth in indicated period (days)									
	1	3	5	7	9	11	13	15	17	21
	Millimeters	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*
W11	5	10	16	25	34	37	42	47	51	57
S1	5	14	22	29	36	38	41	43	44	50
S39	7	18	28	36	40	42	43	45	46	47
S26	8	21	33	45	60	73	81	88	97	(1)
W43	5	11	22	35	47	54	58	63	69	76
NY1	7	20	33	48	65	79	89	102	(1)	(1)
Iowa–11	8	22	35	49	65	81	92	104	(1)	(1)
PS40	7	19	31	45	60	74	83	95	(1)	(1)
PS200	6	19	31	44	60	75	87	99	(1)	(1)

[1] Growth had filled bottle and was beyond measurement.

STABILITY OF STRAINS

Numerous mycelial transfers and single-spore subcultures were made of the isolates being studied. Several saltant strains were isolated from the single-spore cultures. These saltant strains were just as stable through successive transfers as the parent strains. Mutants arising as mycelial sectors occurred most frequently in cultures subject to staling. This was especially true of isolates W11

TABLE 5.—*Growth rates of representative Phoma isolates in 6-ounce bottles, at room temperature and 30° C., on potato-dextrose agar*

[Each value is an average of 8 hypha-tip cultures of each monoconidial isolate]

Isolate	Temperature (° C.)	Growth in indicated period (days)										
		6	8	10	12	14	16	18	22	27	33	51
		Millimeters	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*
W11	Room	6	16	28	39	51	61	69	85	97	104	[1]
	30°	4	8	10	13	16	19	21	25	27	30	34
S1	Room	10	22	31	39	44	50	54	63	72	79	[1]
	30°	3	6	8	10	14	19	23	27	31	35	41
S26	Room	22	39	53	67	82	96	[1]	[1]	[1]	[1]	[1]
	30°	5	7	8	10	13	16	18	21	24	29	39
NY1	Room	23	39	53	66	80	91	98	[1]	[1]	[1]	[1]
	30°	24	39	46	50	53	54	57	60	65	68	77
Iowa-11	Room	25	42	57	71	84	97	[1]	[1]	[1]	[1]	[1]
	30°	27	45	58	67	74	77	80	83	87	94	[1]
PS40	Room	25	40	52	65	80	93	[1]	[1]	[1]	[1]	[1]
	30°	30	48	62	73	87	97	[1]	[1]	[1]	[1]	[1]

[1] Growth had filled bottle and was beyond measurement.

and S1 growing at 30° C. Practically always the saltants grew faster than the parent strains. Growth characters on potato-dextrose agar of some of the saltant strains are as follows:

W11M1.—Fast-growing, white sector that arose at 30° C. Outline of mycelial mat regular but feathery; aerial mycelium abundant, white, with conspicuous cottony balls; pigment beneath mat slight, pinkish; at room temperature sporulation moderate and pycnidia very large, angular, and compound.

W11M2.—Fast-growing sector that arose at 30° C. At room temperature outline of mycelial mat regular; pycnidia abundant, small, black; growth resembling that of W11. At 30° growth faster; pycnidia moderately abundant, small, black; pigment beneath mat greenish black; growth very different from that of W11.

W11M3.—Fast-growing, white sector that arose at room temperature. Mycelium white, mostly submerged; pycnidia abundant, small, with thin, light-colored walls; spore discharge heavy, giving entire surface a bright-pink color; distinctly different from other isolates.

W11M4.—Fast-growing, white sector that arose at 30° C. Pycnidia scant, small, black; differing from W11M1 in almost complete absence of sporulation at room temperature.

W33M2.—Fast-growing, white sector that arose from W33, a sister culture identical with W11, at room temperature; almost identical with W43 and Iowa-11.

W33M3.—Yellow-tan sector that arose at room temperature. Mycelium almost entirely submerged; pycnidia none; sclerotiumlike bodies yellow, 0.25 to 0.5 mm. in diameter; medium pigment yellow.

W33M5.—Fast-growing, white sector that arose at room temperature; identical with W33M2.

S1M1.—Fast-growing sector that arose from staled culture at room temperature; identical with S26.

S1M1M1.—Fast-growing, fleshlike, white sector. Mycelial mat dense, white, ultimately pinkish; sporulation and pigmentation slight. Growth of this type in many S cultures, often overrunning cultures that were untouched for several months.

S1M3.—Fast-growing, white sector that arose in staled culture at 30° C. Growth rates same at 30° and room temperature; aerial mycelium pinkish white; sporulation none; with age very similar to S1M1M1.

S1M4.—Fast-growing, white sector that arose at 30° C. Mycelium with cottony balls like that of W11M1; pycnidia very large, compound, like those of W11M1; very distinct from all other S cultures.

S39M1.—Sector that arose in staled culture at 30° C.; mycelium abundant; pycnidia none.

S26M1.—Fast-growing, white sector that arose in staled culture at 30° C.; mycelial pigment yellow green.

S26SS1.—One of 10 single-spore transfers; identical with S1.

S6M1.—Sector that arose as a fleshlike, white growth in S6, a sister culture identical with S1, at room temperature; identical with S1M3.

S6M1M1.—Sector that arose at room temperature; identical with S26.

Iowa-11M1.—Sector that arose at room temperature; mycelium abundant; pycnidia none; pigment none.

Iowa–11SS1.—One of 10 single-spore transfers, indistinguishable from W11 at room temperature, but with 3 times as much growth at 30° C.

Other saltations that occurred are not listed here. The fleshlike types were recovered particularly from the S isolates. Of the original 200 single-spore isolates of the W series more than 60 changed from the slow-growing, small-pycnidiate type to the fast-growing, large-pycnidiate Iowa–11 type during a 7-month incubation in test tubes without transfer. One changed until it was identical with W11M3; 1 was identical with W33M3; 2 were identical with W11M4; and others showed intermediate rates of growth and degrees of sporulation. Very similar changes occurred in the S series during the 7-month period; many of the S1 type changed to faster growing forms. One recovered was identical with S1M3; 1 was identical with S1M4; and several were of the S26 type. These data seem to indicate that slow-growing forms frequently change to fast-growing types when conditions for growth are unfavorable. During the 7 months without transfer the cultures had dried down considerably.

It may be noted that some of the different groups set up on page 116 have been found to be linked together by saltation. Thus, the fast-growing type of group 2 A was derived from the slow-growing type of group 1 A in W33M2 and W33M5. Similarly, group 2 B was derived from group 1 B in S1M1 and S6M1M1. Also group 1 A was derived from group 2 A in Iowa–11SS1, and group 1 B was derived from group 2 B in S26SS1. In addition, several distinct forms such as W11M1, W11M3, S39M1, and Iowa–11M1 were isolated.

No sectoring was ever observed in isolate PS40.

MORPHOLOGY OF STRAINS

In table 6 are given the sizes of conidia of various representative isolates. It can be seen that there was relatively little difference in the widths but that differences did occur in lengths. The isolates obtained from the East (W11, Iowa–11, and NY1) did not differ much in spore size; nor did the isolates of the S series (S1, S26, and S39). However, conidia of the S group were noticeably shorter than those of the eastern group. Conidia of isolate PS40 were still shorter, but their lengths were nearer those of the S isolates. The close agreement of the sizes of the conidia of the S isolates might suggest their origin to be somewhat removed from that of the isolates from the East.

The sizes and the types of the pycnidia are given in table 7. The isolates were not grouped for pycnidial size as they were for spore size. A great diversity of pycnidial size and shape occurred among the isolates. Although pycnidia of a single isolate varied considerably in size and shape, they were regular enough to be characteristic; W11 and S1 always produced small pycnidia, and Iowa–11 and NY1

always produced large ones. When a slow-growing, small-pycnidiate form gave rise to a fast-growing type, there was a corresponding change in the size of the pycnidia (for example, W33M2, W33M5, and S1M1).

TABLE 6.—*Measurements of conidia of representative Phoma isolates grown for 2 weeks on potato-dextrose agar*

[Measurements of 100 spores except as indicated]

Isolate	Width			Length		
	Average	Maximum	Minimum	Average	Maximum	Minimum
	μ	μ	μ	μ	μ	μ
W11 [1]	1 69	2 50	1.08	4.34	5 83	3.25
Iowa-11	1.67	2 08	1 08	4 39	5.83	3 33
NY1 [2]	1 73	2 33	1.17	4 41	5.83	3 33
S1	1.66	2.08	1.25	4 08	5.50	3 35
S39	1 62	2 33	1.00	4 07	5.87	3 25
S26	1 66	2 50	1 25	4 09	5 91	3.17
PS40	1.62	2.08	1.25	3.97	5 80	3 08

[1] 150 spores measured.
[2] 50 spores measured.

TABLE 7.—*Sizes and types of pycnidia of various Phoma isolates and their saltants grown on potato-dextrose agar at room temperature*

[Measurements and observations on 100 pycnidia of each isolate]

Isolate	Average width	Description of pycnidial type
	μ	
W11	179	Size and shape rather regular (round to broadly flask-shaped with broad base, slightly beaked and slightly papillate), usually simple, but sometimes compound, decidedly black; spore masses readily discharged
W11M1	650	Regular and roundish at first, but becoming irregular and angular with numerous fingerlike protuberances thought to be small pycnidia, spores not readily discharged, but large masses released by crushing.
W11M3	156	Shape very similar to that of W11, but size noticeably smaller and wall color noticeably lighter; pycnidia made pinkish by spore masses even before discharge; spore masses discharged profusely.
S1	139	Size and shape similar to those of W11 except smaller and slightly more flask-shaped.
S1M4	744	Indistinguishable from W11M1.
S26	236	Size rather irregular (some flask-shaped like those of S1; others roundish like those of Iowa-11), lighter colored than those of S1; spore discharge slight.
S39	633	Sclerotiumlike bodies large, roundish, smooth, hard, black, with slight spore discharge at different points, probably indicating presence of pycnidia that developed peripherally in them.
Iowa-11	385	Size and shape in general rather regular (large, roundish), occasionally beaked, irregular, and compound; lighter in color and more papillate than those of W11; spore masses readily discharged.
W43	332	Almost same as Iowa-11.
PS40	340	Size and shape very irregular (some very small, flask-shaped like those of W11 others large, round like those of Iowa-11, distinctly beaked); many simple, others compound, black.

PATHOGENICITY OF STRAINS

Numerous tests were made to determine the pathogenicity of representative isolates and their saltants. One method of inoculation was to soak seeds of the host species being tested in a spore suspension for 48 hours before planting. Within 2 weeks the emerged seedlings developed cotyledonary lesions and severe damping-off. Isolates PS40, PS200, and C1 were easily distinguishable from all others by this method. The cotyledonary lesions caused by these isolates

appeared 3 to 5 days earlier than those caused by other isolates and were of a distinctly different type; usually they were marginal and chocolate brown from the beginning and enlarged slowly; the pycnidia produced on the lesions were few, relatively large, and more brown than black. Cotyledonary lesions produced by all other isolates were almost identical and appeared as shrunken, dark-green areas, which rapidly enlarged and became thickly studded with pycnidia before the lesion surfaces became dark. Results obtained on radish (*Raphanus sativus* L.), Chinese cabbage, cabbage, turnip, rutabaga, kale (*Brassica oleracea* var. *acephala* DC.), and brussels sprouts (*B. oleracea* var. *gemmifera* Zenker) in one such test are given in table 8. It is apparent that isolates S1, S39, and S26 were generally less virulent than the others.

TABLE 8.—*Results of inoculating seeds of various cultivated crucifers with representative isolates of Phoma*

Isolate	Approximate portion of seedlings of indicated species killed						
	Radish	Chinese cabbage	Cabbage	Turnip	Rutabaga	Kale	Brussels sprouts
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
W11	40	80	80	60	100	100	100
S1	40	40	60	40	100	60	40
S39	20	40	40	20	60	60	40
S26	20	20	20	20	40	40	40
NY1	80	100	100	40	100	100	80
W43	60	80	80	60	100	100	100
PS40	80	100	100	40	60	100	100

Inoculations of older plants were made by spraying the leaf surfaces with spore suspensions and by sponging leaves previously sprinkled with powdered carborundum with a ball of absorbent cotton dipped in a spore suspension. The carborundum removed some of the bloom and thus increased the wetting of the leaves. Stem inoculations were made by dipping roots of small seedlings in a spore suspension before transplanting and by pouring spore and mycelial suspensions on the surfaces of soil in which plants were growing, with and without wounding the stems. In table 9 are given the results obtained with large plants of cauliflower (*Brassica oleracea* var. *botrytis* L.), broccoli (*B. oleracea* var. *botrytis* L.), rape (*B. napus* L.), and other species.

On leaves of brussels sprouts isolates S1, S26, S39, and PS40 produced very similar effects. Black necrotic flecks surrounded by a conspicuous chlorotic halo developed at the infection sites. These flecks enlarged very slowly and rarely exceeded 5 mm. in diameter, but occasionally they developed into extensive necrotic areas. Sporulation was slight. Isolates W11, Iowa–11, W43, and NY1 produced much more extensive necrosis than the other group, causing complete blighting of the leaves. Lesions did not develop the conspicuous halo produced by the other strains. Sporulation was slight. On stems of brussels sprouts the isolates differed from each other only in virulence and in degree of sporulation. All produced lesions typical of blackleg, but none was as virulent as on cabbage or cauliflower.

TABLE 9.—*Results of inoculating leaves and stems of various cultivated crucifers with representative Phoma isolates and their saltants*

[+, slight virulence; ++, moderate; +++, severe; ++++, very severe]

Isolate	Virulence of symptoms on host indicated											
	Cabbage		Brussels sprouts		Cauliflower		Broccoli leaves	Turnip leaves	Rutabaga leaves	Kale leaves	Chinese cabbage leaves	Rape leaves
	Leaves	Stems	Leaves	Stems	Leaves	Stems						
W11	++++	++++	++++	+++	+++	+++	++++	+	++++	+++	++++	++
W11M1	++	------	++	++	++	++	+++	------	------	++	------	------
W11M2	+++	------	+++	++	+++	+++	++++	------	------	+++	------	------
W11M3	+	------	+	+	+	+	++	------	------	+	------	------
W11M4	++	------	++	+++	+	++	+++	------	------	+	------	------
S1	+++	+++	++	++	++	++	++	++	++++	+	++++	++
S1M1	------	------	------	++	++	++	++	------	------	+	------	------
S1M3	------	------	------	+	+	+	------	------	------	------	------	------
S1M4	------	------	------	++	+	++	++	------	------	+	------	------
S26	+++	+++	++	++	++	++	------	+	+++	------	++++	++
S26M1	------	------	------	++	------	------	------	------	------	------	------	------
S39	+++	+++	++	++	++	++	------	+	++++	------	+++	++
S39M1	------	------	------	+	------	------	------	------	------	------	------	------
Iowa-11	++++	++++	++++	+++	+++	+++	++++	+	++++	+++	++++	++
W43	++++	++++	++++	+++	+++	+++	++++	+	++++	+++	++++	++
NY1	++++	++++	++++	+++	+++	+++	++++	+	++++	+++	++++	++
W307	------	++++	------	------	+++	+++	++++	------	------	+++	------	------
PS40	+++	++	++	+++	++	++	++	------	++	++	+++	+
C1	+++	++	------	+++	++	++	------	+	++	------	------	------

On leaves of cabbage, cauliflower, and broccoli symptoms produced by PS40 were distinct from all others except those produced by W11M3, which they closely resembled. Numerous black necrotic flecks developed in 6 to 10 days. These small lesions enlarged very little before the leaves became chlorotic and abscised. Sporulation was scant to none. Occasionally a few lesions enlarged and developed light centers and pycnidia as in nature. Several weeks were required to reach this stage. If the spore load used was not very light, the leaves fell off before this stage of development was reached. On cabbage and cauliflower isolate C1 was identical with PS40. Isolates W11, W11M1, W11M2, NY1, Iowa–11, W43, and W307 produced identical symptoms as circular lesions which rapidly enlarged, coalesced, and caused complete necrosis. Affected tissue collapsed as if killed by steam and was covered with pycnidia while still dark green. Eventually the lesion surfaces became ashen gray. Isolates S1, S26, S39, S1M1, and S1M4 produced similar symptoms but were generally less virulent than the eastern isolates. Isolate W11M3 was very much less virulent than W11 and produced symptoms more like those of PS40. Typical symptoms produced on broccoli leaves by isolates W11, PS40, and W11M3 are shown in figure 2.

On the stems of large cabbage plants (fig. 3) all the isolates tested produced lesions typical of blackleg. Again PS40 and the S isolates were less virulent than W11, NY1, and Iowa–11. Isolates of the second group often killed the plants.

On leaves of Chinese cabbage isolate PS40 was distinct from all others tested (fig. 4). Numerous black flecks appeared 2 to 4 days before symptoms were produced by other isolates. These angular lesions developed very slowly and reached a diameter of not more than 3 to 5 mm. before the leaves became yellow and abscised.

FIGURE 2.—Symptoms produced on broccoli leaves by three *Phoma* isolates: *A*, W11; *B*, PS40; *C*, W11M3. Note abundance of pycnidia on lesions produced by W11 and their absence on others. Note also the similarity of the lesions produced by PS40 and W11M3.

FIGURE 3.—Symptoms produced on stems of large cabbage plants by various *Phoma* isolates: *A*, Iowa–11; *B*, NY1; *C*, W11; *D*, uninoculated; *E*, S26; *F*, S39; *G*, S1; *H*, PS40. Note that the lesions produced by the eastern isolates (Iowa–11, NY1, and W11) are more severe than those produced by the western isolates (S26, S39, S1, and PS40).

FIGURE 4.—Symptoms produced on Chinese cabbage leaves by *Phoma* isolates: *A*, Iowa-11, and *B*, PS40. Note abundance of pycnidia on lesions produced by Iowa-11 and the flecking only produced by PS40.

Sporulation was scant. When a very light spore load was used, however, lesions reached diameters of 10 to 20 mm., became brown, and were covered with pycnidia. When the infections occurred on the fleshy petiole, necrosis often extended to the leaf axil and lesions formed on the stems. These symptoms approximated those occurring in nature. Isolates S1, S26, S39, W11, NY1, and Iowa-11 caused small flecks, which rapidly enlarged and became covered with pycnidia before extensive necrosis was evident. There was little difference in the symptoms produced by these isolates.

On turnip leaves all isolates produced only pin-point necrotic flecks. These lesions did not develop beyond the fleck stage, and no abscission resulted. No isolate sporulated on this host. On rutabaga, however, all isolates produced extensive infection and brown necrotic lesions up to 20 mm. in diameter. All isolates produced similar effects on this host, but PS40 and C1 were less virulent than the others.

In the spring of 1945 rutabaga plants in midbloom were sprayed with spore suspensions of S1, S26, S39, W11, NY1, Iowa-11, and PS40. At biweekly intervals during the remainder of the blossoming period the plants were watered with a hose nozzle to simulate rain-

storm conditions. At maturity the pods were examined and lesions typical of those caused by *Phoma lingam* were found on pods inoculated with all isolates (fig. 5). The lesions produced by isolate PS40 differed from the others only in producing fewer pycnidia. Seed from infected pods was accidentally destroyed before a test for seed transmission could be made.

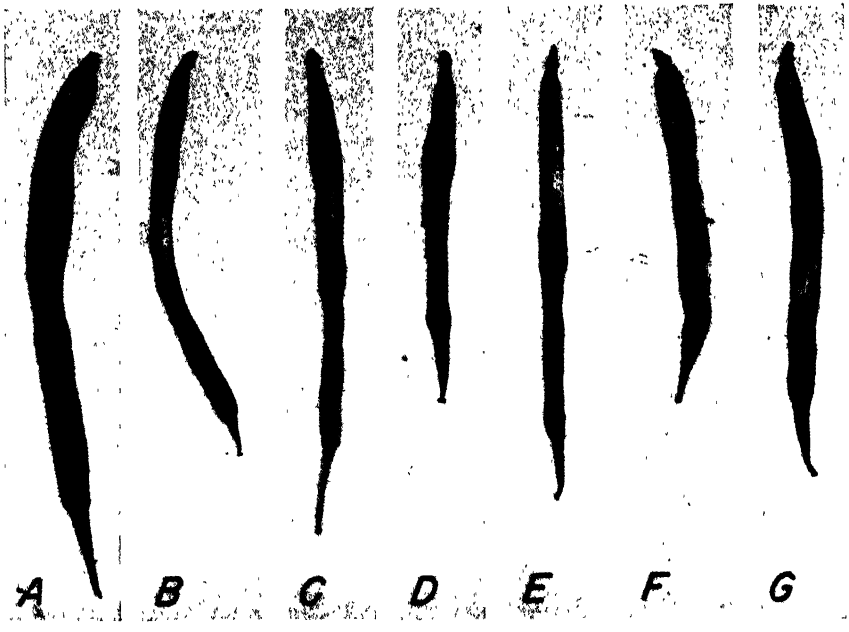

FIGURE 5.—*A*, Healthy rutabaga seed pod. *B–G*, Lesions produced on rutabaga seed pods by various *Phoma* isolates: *B*, S1; *C*, S26; *D*, S39; *E*, W11; *F*, NY1; *G*, PS40. Note similarity of the lesions.

Thus, in the pathogenicity tests recorded isolates of the S series and PS40 were nearly always found to be less virulent than isolates W11, NY1, and Iowa–11. Isolate W11M3, a saltant of W11, was much less virulent than W11 and more like PS40 in the type of symptoms that it produced.

DISCUSSION

The disease caused by a *Phoma* species described in this paper has been observed to occur regularly in the seed-growing district of the Puget Sound section of Washington. Only traces have been found in cabbage fields, where it caused scattered leaf lesions and occasional stalk rots of seed plants. Typical blackleg of cabbage, however, has not been observed in this district. The same species of *Phoma* has also been observed to infect rutabaga, turnip, and Chinese cabbage seed plants in the Puget Sound section. On Chinese cabbage it has been more virulent than on other hosts. On this host lesions commonly occur on leaf petioles from which they spread to the leaf axil, and stalk infections result. As flower heads emerge from leaf axils,

they become infected and severe blighting of florets results. Lesions on stalks at the ground level and above are common and cause stalks to break over. Sporulation on lesion surfaces is abundant. Lesions have not been found to occur naturally on seed pods, and it is believed that the dry season during which pods develop and mature checks the spread to seed pods. That the Puget Sound *Phoma* can infect pods was shown by experimental work. Although in the threshing process seed surfaces could come in contact with spores, it is not believed that this would result in much seed transmission, since naked spores do not remain viable long. It was not determined whether this strain infected seeds internally as does the *Phoma* that causes cabbage blackleg.

How the Puget Sound *Phoma* lives over from season to season was not determined; but, since there is neither much crop rotation nor any crucifer-free period in the production of cruciferous seed crops in the Puget Sound section, overwintering would not be a factor in the disease cycle. The rather weak appearance of this fungus on cabbage in nature plus its widespread occurrence might indicate that it occurred commonly as a soil-borne saprophyte and less commonly as a parasite. Its extreme pathogenicity on certain hosts in the greenhouse would indicate, however, that it is not necessarily a facultative parasite. This is further borne out by its virulence on Chinese cabbage in nature. In view of the long and intensive culture of crucifer seed crops in the Puget Sound section, it seems more probable that this *Phoma* is carried from crop to crop by plant debris and living hosts.

The *Phoma lingam* that causes typical blackleg of cabbage was shown to be very variable, and several cultural strains of it were isolated. These strains differed markedly in growth type and rate and slightly in pathogenicity and morphology. Saltation occurred frequently and was found to link the strain groups together. Unfavorable growing conditions apparently increased the frequency of saltation. It is very probable that further study would reveal numerous other types. In view of the wide variability of *P. lingam* it seems logical to conclude that the Puget Sound isolates, the Oregon isolates, and the California isolate, which fall within that range of variability in morphology, physiology, and pathogenicity, are variants of *P. lingam*. Although the organism causing dry rot of rutabaga in New Zealand and Europe was not examined by the writer, it seems logical to conclude that it also belongs to *P. lingam* since the results obtained by Cunningham (*2*), Hughes (*6*), and Buddin (*1*) indicate that it falls within the range of variability of this species.

It may be well to point out, however, that, although the isolates of *Phoma* from the Puget Sound section were within the range of variation of *P. lingam*, they showed certain points of difference. They, with the isolate from California, were distinct from all other isolates in the production of a water-soluble, yellow or brown pigment in the media, in a yellow to tannish-brown pigmentation of mycelium, and in the coarser appearance of the mycelium in culture. The Puget Sound isolate PS40 was apparently stable in culture, whereas isolates from the East and from Oregon were more or less variable. The conidia of PS40 were shorter than the spores from any other isolate.

Isolates from the Puget Sound section and from California were also distinct from other isolates in the type of lesion produced on cotyledons of seedlings grown from inoculated seeds and in the symptoms produced on inoculated leaves of older cabbage plants.

In view of these findings it is still a question whether the strain of *Phoma* found in the Puget Sound section would cause typical blackleg on cabbage in sections favorable to the disease even if it were transmitted there by seed from the Puget Sound section. Although it may be regarded as a strain of *P. lingam*, its potential danger as a harmful seed-borne pathogen on cabbage has yet to be demonstrated. Even though nothing in this study indicates that cabbage seed grown in the Puget Sound section is not free from blackleg, it should be pointed out that the Pacific coast seed-producing section cannot be regarded as free from all strains of *P. lingam*. Moreover, the occurrence of a mild strain of what appears to be *P. lingam* in the important Puget Sound section and of other strains in the Willamette Valley of Oregon is reason for emphasizing that the possibility of seed contamination and transmission in that section should not be overlooked.

SUMMARY

A *Phoma* has been found to occur regularly as a minor leaf spot and stalk rot pathogen of seed plants of cabbage, Chinese cabbage, rutabaga, and turnip in the Puget Sound section of Washington. Experiments showed that it is widely pathogenic on other cruciferous plants. It produces typical blackleg symptoms on *Brassica oleracea* in greenhouse tests.

In determining the relation of this fungus to *Phoma lingam*, the cabbage blackleg organism, a survey of variability in the latter was made. Several cultural strains that differed in type and rate of growth, degree of staling, and amount of sporulation were established by single-spore technique. In a comparative study of these strains numerous saltants that tended to link the strains together were isolated. Cultural studies of the variant strains indicated that the Puget Sound isolates differed from those from typical blackleg material in absence of staling, in production of water-soluble yellow to brown pigment in both medium and mycelium, and in a coarser mycelium.

The Puget Sound isolates produced symptoms that differed from those produced by isolates from typical blackleg material in type and virulence and were thus separable from the latter.

Only slight differences in conidial size were found among the strains, but marked differences in size and shape of pycnidia occurred.

The Puget Sound strain of *Phoma* appeared to fall within the range of variability in morphology, physiology, and pathogenicity of *P. lingam* and is considered a variant of it.

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ELIMINATION AND RETENTION OF POLLEN STERILITY IN POTATO IMPROVEMENT [1]

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INTRODUCTION

Improvement in the potato (*Solanum tuberosum* L.) has generally been accomplished through sexual breeding. Since pollen sterility interferes with sexual breeding, potato improvement would be facilitated if this factor were eliminated from the breeding material. Pollen sterility is, however, a desirable character in a potato variety. Complete pollen sterility when present prevents fruiting in this normally self-pollinated crop. Bartholdi (*2*) [2] secured a significant increase in yield of tubers by removing flower buds from a self-fruitful (fertile-pollen) variety, and a significant decrease in yield by pollinating a nonfruiting (sterile-pollen) variety and producing fruit development. Thus pollen fertility is essential for potato improvement but undesirable in a variety intended for cultivation. More complete information concerning the inheritance of pollen sterility would aid in the formulation of a breeding procedure that would reduce the interference from pollen sterility to a minimum and yet provide a means for its retention and utilization.

Pollen sterility in potato varieties may be complete (sterile-pollen varieties) or partial (fertile-pollen varieties). The fertile-pollen varieties can be further classified according to the percentage of pollen that is stainable with acetocarmine (*9*). A description of the breeding behavior of a number of sterile-pollen and fertile-pollen varieties selected from the breeding material of the Minnesota Agricultural Experiment Station will be presented, together with a discussion of the significance of the results in respect to breeding procedure for the improvement of the potato.

CHARACTERISTICS OF STERILE-POLLEN AND FERTILE-POLLEN PLANTS

The breeding behavior of varieties was analyzed with respect to pollen sterility by classifying their progenies into sterile-pollen and fertile-pollen plants.

Fertile-pollen plants were characterized by abundant pollen, with the stainable grains invariably round and plump, 35 to 50 microns in size, and the nonstainable grains uniformly smaller and resembling collapsed spheres, and by the absence of pollen grains of the type

[1] Received for publication March 11, 1946.
[2] Italic numbers in parentheses refer to Literature Cited, p. 145.

indicative of irregular meiosis. Examples of pollen from fertile-pollen plants are shown in figure 1.

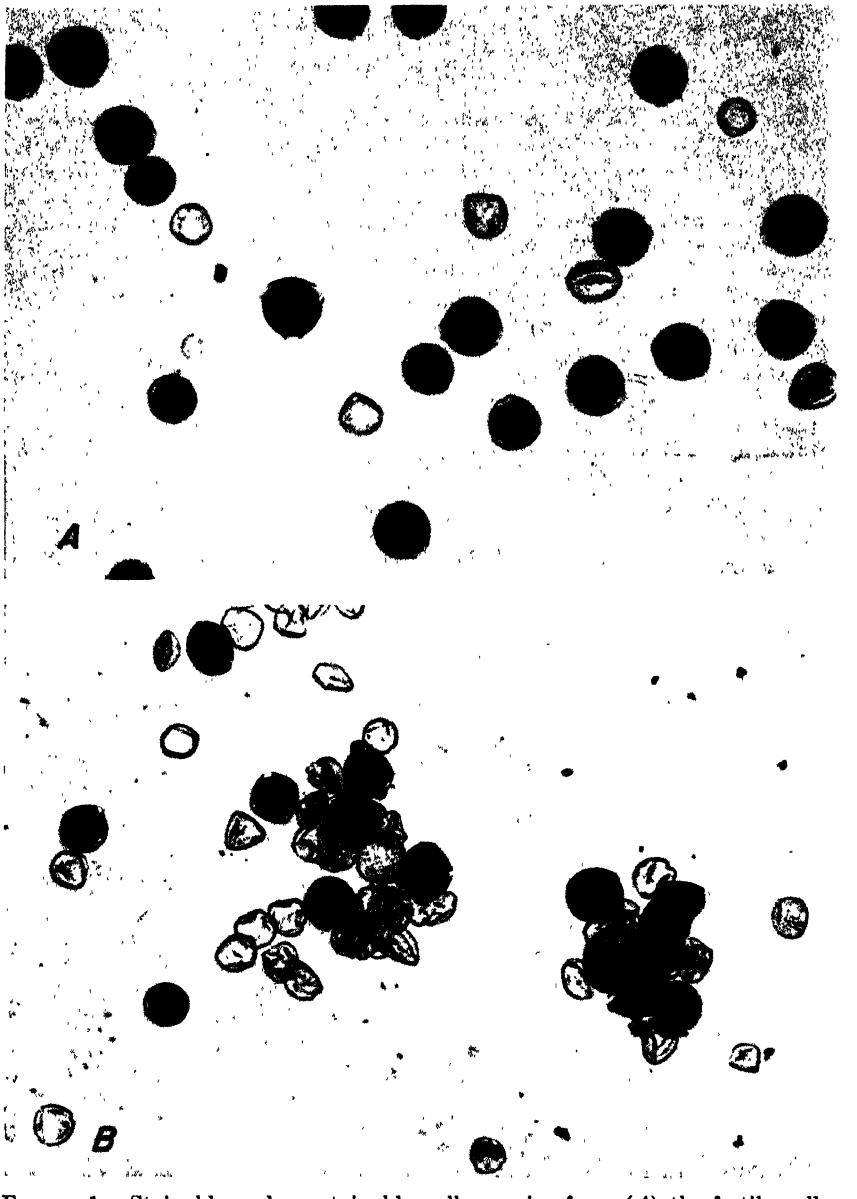

FIGURE 1.—Stainable and nonstainable pollen grains from (*A*) the fertile-pollen clone 15–2, which had 76.9 percent of stainable pollen, and (*B*) from the fertile-pollen clone 13–1, which had 24.9 percent of stainable pollen. ×365.

Sterile-pollen plants were characterized by the absence of normal appearing stainable pollen grains (fig. 2) and by the presence of pollen indicative of irregular meiosis. The pollen of sterile-pollen plants ranged in quantity from scant to abundant; the grains ranged in size from one-fourth to twice the size of normal stainable grains, and were irregularly shaped (fig. 2). Further indications of irregular meiosis

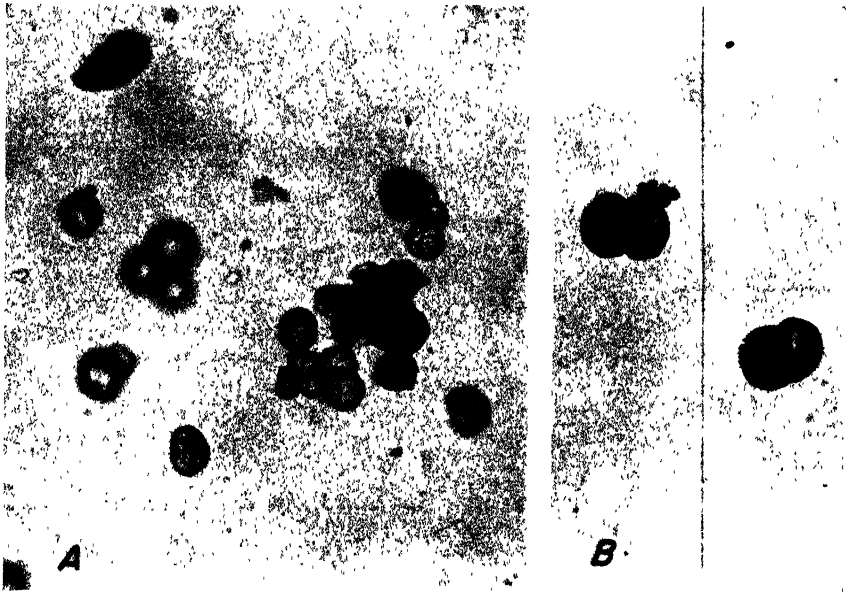

FIGURE 2.—*A*, Pollen from the sterile-pollen clone 77 9 having 5.6 percent stained pollen; *B*, pollen from the sterile-pollen clone 77–7, having 2.8 percent stainable pollen. X365.

were the presence of large grains with thick rugose walls of the sort described by Fukuda (*6*) as having been dreived from unreduced pollen-mother cells. Grains having two or more distinct sections or cells (fig. 2) were present at times. Three-, four-, and five-sectioned grains were less common.

Occasionally in sterile-pollen plants a few grains and one or more sections of a multisectioned grain were stainable, but these could be distinguished from normal stainable grains by various irregularities in shape and size. Scantiness of pollen was associated with a reduction in size of both single and multisectioned grains. A few plants contained neither stainable pollen grains nor abnormalities indicative of irregular meiosis.

The complete pollen sterility of sterile-pollen plants is, according to Arnason (*1*) and Longley and Clark (*10*), the result of the failure of normal microspore formation during meiosis. This view is supported by numerous meiotic irregularities observed in sterile-pollen plants (*1*, *3*, *5*, *6*, *7*, *10*). The more common meiotic irregularities observed were: (1) Failure of the chromosomes to pair; (2) lagging of the chromosomes on the spindle; and (3) failure to complete the normal reduction-division process.

According to Arnason (*1*) and Longley and Clark (*10*), the partial sterility of the fertile-pollen plants is conditioned, after what appears to be a normal meiosis, by microspore abortion, which frequently occurs after the spores have reached normal pollen-grain size.

SAMPLING THE MATERIAL

In 1938 and 1939 studies were made at both University Farm (St. Paul) and Castle Danger, Minn., on the north shore of Lake Superior, after which the studies were conducted only at Castle Danger. Since it is well known that the percentage of stainable pollen varies with changes in the environment (*4*, *9*), the studies were made only in periods where flowering and fruit setting appeared to be proceeding normally. Under these conditions, very little variation in stainable pollen resulting from differences in season or location was obtained.

The percentage of stainable pollen was relatively uniform on identical material grown in both years and at two locations. This is shown by the percentage of stainable pollen observed in four selections for each of 2 years and two locations (table 1). The four selections were chosen because they were known to approximate the range in percentage of stainable pollen found in the fertile-pollen plants in the breeding material. No significant difference in percentage of stainable pollen due to either location or years, or both, is shown in table 1. The differences between the clones were fairly consistent for all four tests.

TABLE 1.—*Percentage of stainable pollen in 4 fertile-pollen parent clones at University Farm and Castle Danger in 1938 and 1939*

Parent clones	Stainable pollen in clones at indicated locations and seasons				Mean [1]
	1938		1939		
	University Farm	Castle Danger	University Farm	Castle Danger	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
15-2	76.1	83.0	78.7	74.3	78.5
5-1	67.2	76.0	75.3	73.3	73.0
12-7	57.2	50.9	60.3	34.7	50.8
13-1	29.2	26.5	20.6	23.5	25.0
Mean [1]	57.4	59.6	58.7	51.5	

[1] Mean difference necessary for significance, 9.44 percent.

The mean percentages of stainable pollen obtained in sexual progenies at University Farm and at Castle Danger are presented in table 2. Four selfed and three crossed seedling progenies were used in this study. It will be noted that there was no significant difference due to location in the mean percentage of stainable pollen. These results suggest that limiting the studies to periods favorable to blooming tended to make the data obtained in different seasons and at different locations fairly comparable. Since a careful study of the data failed to indicate any influence of season or location on percentage of stainable pollen, these factors have been omitted in the presentation of later data.

BREEDING BEHAVIOR OF PLANTS

RECIPROCAL CROSSES

In the present study five sets of reciprocal crosses were made between parents which differed significantly in percentage of stainable pollen. The number of sterile-pollen and fertile-pollen plants and the means in percentage of stainable pollen in the fertile-pollen portion of the progenies of the reciprocal cross are given in table 3. In all five cases, the two progenies of reciprocal crosses differ significantly in the proportion of sterile-pollen to fertile-pollen plants and in the mean percentage of stainable pollen in the fertile pollen-plants. The differences between the two progenies of reciprocal crosses is associated with a similar difference in the stainable pollen of the female parents.

TABLE 2.—*Mean percentages of stainable pollen in the fertile-pollen plants in duplicate samples of selfed and crossed progenies at University Farm and Castle Danger in 1939*

Progenies	Stainable pollen in progenies [1] at—		Progenies	Stainable pollen in progenies [1] at—	
	University Farm	Castle Danger		University Farm	Castle Danger
	Percent	*Percent*		*Percent*	*Percent*
13-1 selfed	46 5±9 7	39 7±4 1	75-5×15-2	67 4±4 0	61 3±8 5
5-1 selfed	32 7±6 0	30 2±7 3	15-2×5-1	68 1±2 1	66 2±4 8
75-5 selfed	43 8±4 3	50 4±11 7			
15-2 selfed	66 3±3 0	56 4±6 3	Mean	49 4	47 9
13-1×5-1	20 9±6 0	31 3±10 7			

[1] In fertile-pollen plants.

TABLE 3.—*Sterile-pollen and fertile-pollen plants obtained in 5 reciprocal crosses between parents differing in percentage of stainable pollen*

Parents	Stainable pollen in female parent	Sterile-pollen plants	Fertile-pollen plants	Stainable pollen in progeny
	Percent	*Number*	*Number*	*Percent*
13-1 × 15-2	24 9	28	39	20 7
15-2 × 13-1	76 9	5	40	33 6
12-7 × 5-29	56 3	41	10	8 5
5-29 × 12-7	71 8	5	48	48 6
17-2 × 11-1	32 8	30	79	32 5
11-1 × 17-2	90 0	1	150	73 9
80-7 × 15-2	56 4	7	9	23 6
15-2 × 80-7	76 9	0	12	61 3
106-55 × 5-2	26 1	62	50	22 6
5-2 × 106-55	74 7	12	106	53 2

In all five reciprocal crosses female parents with a relatively low percentage of stainable pollen gave relatively low proportions of fertile-pollen plants as compared with female parents that had a relatively high percentage of stainable pollen. It is evident from the results of the five reciprocal crosses that parents with a low percentage of stainable pollen transmit more pollen sterility as female parents than as male parents. These results are in harmony with those of Salaman and Lesley (*11*), who found that the two progenies of a reciprocal cross were distinctly different in respect to pollen sterility and in fruit setting.

BREEDING BEHAVIOR OF PLANTS WHEN SELFED AND WHEN USED AS FEMALE PARENTS

The behavior of reciprocal crosses suggested that the factors responsible for sterile-pollen plants tended to be eliminated in the aborted pollen. Thus, with the reduced transmission of these factors through the pollen, the character of the progeny with respect to pollen sterility would be mostly determined by the breeding behavior of the female parent. If this were true, one might expect only slight differences in pollen sterility between the progenies of an individual when selfed and when used as a female parent in crosses irrespective of the male parent.

In table 4 are given the number of sterile-pollen and fertile-pollen plants in both the crossed and selfed progenies of nine female parents. For five of these nine, the crossed and selfed progenies do not vary widely from each other. The crossed progeny from 80–7 has a slightly higher proportion of sterile-pollen plants than its selfed progeny. The crossed progenies from 12–7, 21–2, and 13–1 have a much higher proportion of sterile-pollen plants than their selfed progenies. An association between the amount of stainable pollen in the parent and the proportion of fertile-pollen plants in the progeny is indicated, except for the crossed progenies of parents 21–2 and 12–7. Thus, for two of the nine parents, the breeding behavior in crosses did not seem to be closely related to the behavior when selfed or to the stainable pollen in the parent. This fact suggests that in some crosses the male parent may have a significant influence on the pollen sterility of the progeny.

CROSSES SHOWING THE RELATIVE INFLUENCE OF MALE AND FEMALE PARENTS

The possible influence of the male parent was studied by comparing crossed progenies having the same female parent but different male parents. The results obtained by crossing each of eight female parents with a number of different male parents are presented in table 5. In general, the progenies within the groups having the same female parent tend to be alike in the proportion of sterile-pollen to fertile-pollen plants. Nevertheless, there are some differences between the progenies of a group that are worth noting. In table 5, for example, the crossed progenies from male parents 12–7 and 80–7 had a higher proportion of fertile- to sterile-pollen plants than the progenies from the other male parents in the same group, and, with one exception, these other male parents had a higher percentage of stainable pollen. The difference between the male and female breeding behavior of selections 12–7 and 80–7 was also apparent when reciprocal crosses were made and highly contrasting progenies were obtained (table 3).

As indicated by the data in tables 3–5, the percentage of stainable pollen in a clone and its breeding behavior on selfing and as a female parent in crosses was not related to its breeding behavior when used as a male parent. The difference between the male and female breeding behavior of selections 12–7 and 80–7 might indicate that the factors for the relatively high proportion of sterile-pollen plants in their crossed progenies when used as female parents were not transmitted through the pollen of selections 11–25, 15–2, and 75. This explanation assumes that there was some transmission of the factors responsible for sterile-pollen plants through the pollen of some

TABLE 4.—*Sterile-pollen and fertile-pollen plants obtained in the crossed and selfed progenies of 9 female parents with different percentages of stainable pollen*

Female parents	Stainable pollen in parent	Type of progeny	Sterile-pollen plants	Fertile-pollen plants
	Percent		*Number*	*Number*
11-1	90.0	Crossed	0	151
		Selfed	0	38
15-2	76.9	Crossed	6	107
		Selfed	1	43
75-5	72.9	Crossed	0	30
		Selfed	5	43
5-29	71.8	Crossed	5	48
		Selfed	0	7
80-7	56 4	Crossed	13	15
		Selfed	6	13
12-7	56 3	Crossed	41	10
		Selfed	6	11
21-2	49 1	Crossed	43	8
		Selfed	5	10
17-2	32 8	Crossed	30	79
		Selfed	8	19
13 1	24 9	Crossed	57	81
		Selfed	6	12

TABLE 5.— *Sterile-pollen and fertile-pollen plants in crossed progenies having the same female parent but different male parents*

Cross	Stainable pollen in		Sterile-pollen plants	Fertile-pollen plants
	Female parent	Male parent		
	Percent	*Percent*	*Number*	*Number*
15-2 × 5-1	76 9	69 5	1	55
15 2 × 80-7	76 9	56 4	0	12
15-2 × 13-1	76 9	24 9	5	40
80-7 × 15 2	56 4	76 9	7	9
80-7 × 5-1	56 4	69 5	6	6
13-1 × 15-2	24. 9	76 9	28	39
13-1 × 5 1	24 9	69 5	29	24
13-1 × 80 7	24 9	56 4	0	18
77-9 × 75-5	5 6	72 9	18	4
77-9 × 11-25	5 6	72 4	117	3
77-9 × 5-29	5 6	71 8	58	15
77-9 × 12-7	5 6	56 3	26	11
76-1 × 75-5	4 3	72 9	18	4
76-1 × 11-25	4 3	72 4	56	1
76 1 × 5-29	4 3	71 8	26	8
76-1 × 12-7	4 3	56 3	13	6
77-7 × 75-5	2 8	72 9	34	3
77-7 × 11-25	2 8	72 4	52	5
77-7 × 5-29	2 8	71 8	32	3
77-7 × 12-7	2 8	56 3	68	31
77-8 × 75-5	0	72 9	31	0
77-8 × 11-25	0	72. 4	23	1
77-8 × 5-29	0	71 8	36	0
77-8 × 12-7	0	56 3	30	3
Russet Rural × 15-2	0	76. 9	60	13
Russet Rural × 11-25	0	72 4	93	14
Russet Rural × 5-29	0	71 8	109	4

selections. To the breeder, the significant aspect of the breeding behavior of the pollen parents shown in table 5 is that fertile-pollen plants such as 12–7, 13–1, and 80–7 whose percentage of stainable

pollen is low and whose progenies when selfed and when used as female parents in crosses may give a relatively high proportion of sterile-pollen plants, may transmit less sterility through the pollen than male parents having a significantly higher percentage of stainable pollen.

The influence of the male parent on the crossed progeny is, however, relatively small as compared with that of the female parent. The influence of the female parents is shown in table 6, which gives the segregation obtained from different female parents crossed with the same male parent. The largest differences in segregation between comparable progenies of female parents was obtained in groups 1, 4, and 5. These groups contained female parents with a wider range of percentage of stainable pollen than the other groups. An association between the percentage of stainable pollen in the female parent and the proportion of sterile- to fertile-pollen plants in their progenies is indicated in group 1 and to a lesser extent in groups 4 and 5. Some female parents (12–7 and 77–9, group 4) which differed widely in percentage of stainable pollen, when crossed with the same male parent produced progenies with relatively small differences in proportion of sterile- to fertile-pollen plants. Other female plants (80–7 and 21–2, group 5) with no significant difference in stainable pollen produced progenies having wide differences.

TABLE 6.—*Sterile-pollen and fertile-pollen plants in crossed progenies having the same male parent but different female parents*

Group	Cross	Stainable pollen in parent		Sterile-pollen plants	Fertile-pollen plants
		Female	Male		
		Percent	*Percent*	*Number*	*Number*
1	75–5 × 15–2	72.9	76.9	0	30
	80–7 × 15–2	56.4	76.9	7	9
	13–1 × 15–2	24.9	76.9	28	30
	Russet Rural × 15–2	0	76.9	60	13
2	77–9 × 75–5	[1] 5.6	72.9	6	0
	76–1 × 75–5	[1] 4.3	72.9	18	4
	77–7 × 75–5	[1] 2.8	72.9	34	3
	77–8 × 75–5	[1] 0	72.9	31	0
3	77–9 × 11–25	[1] 5.6	72.4	117	13
	76–1 × 11–25	[1] 4.3	72.4	56	1
	77–7 × 11–25	[1] 2.8	72.4	52	5
	77–8 × 11–25	[1] 0	72.4	23	1
	Russet Rural × 11–25	[1] 0	72.4	93	14
4	12–7 × 5–29	56.3	71.8	41	10
	77–9 × 5–29	[1] 5.6	71.8	58	15
	76–1 × 5–29	[1] 4.3	71.8	26	8
	77–7 × 5–29	[1] 2.8	71.8	32	3
	77–8 × 5–29	[1] 0	71.8	36	0
	Russet Rural × 5–29	[1] 0	71.8	109	4
5	15–2 × 5–1	76.9	69.5	1	55
	80–7 × 5–1	56.4	69.5	6	6
	21–2 × 5–1	55.9	69.5	43	8
	13–1 × 5–1	24.9	69.5	29	24
	Jubel × 5–1	20.9	69.5	11	14
6	77–9 × 12–7	[1] 5.6	56.3	26	11
	76–1 × 12–7	[1] 4.3	56.3	13	6
	77–7 × 12–7	[1] 2.8	56.3	68	31
	77–8 × 12–7	[1] 0	56.3	30	3

[1] Sterile-pollen plants.

BREEDING BEHAVIOR OF THE FERTILE-POLLEN PLANTS FROM A CROSS

Twenty-three fertile-pollen plants from a cross were selfed and the progenies examined to determine the proportion of sterile- to fertile-pollen plants. The study was made on a cross of 17–2×11–1 which produced a much higher proportion of sterile- to fertile-pollen plants than its reciprocal (table 3). The results obtained on the selfed and crossed progenies of the parents and on the selfed progenies of 23 F_1 fertile-pollen plants is presented in table 7. This study on the parents

TABLE 7.—*Sterile-pollen and fertile-pollen plants in the selfed, reciprocal crossed, and F_2 progenies of fertile-pollen parents 17–2 and 11–1*

Parents	Sterile-pollen plants	Fertile-pollen plants	Parents	Sterile-pollen plants	Fertile-pollen plants
	Number	*Number*		*Number*	*Number*
17-2 selfed	8	19	17-2 × 11-1-F_2-11	8	19
17-2 × 11-1	30	79	17-2 × 11-1-F_2-12	7	17
11-1 selfed	0	36	17-2 × 11-1-F_2-13	1	5
11-1 × 17-2	1	150	17-2 × 11-1-F_2-14	4	6
17-2 × 11-1-F_2-1	22	15	17-2 × 11-1-F_2-15	4	7
17-2 × 11-1-F_2-2	16	7	17-2 × 11-1-F_2-16	10	8
17-2 × 11-1-F_2-3	10	6	17-2 × 11-1-F_2-17	7	13
17-2 × 11-1-F_2-4	11	7	17-2 × 11-1-F_2-18	8	25
17-2 × 11-1-F_2-5	9	4	17-2 × 11-1-F_2-19	4	14
17-2 × 11-1-F_2-6	9	6	17-2 × 11-1-F_2-20	3	10
17-2 × 11-1-F_2-7	5	4	17-2 × 11-1-F_2-21	3	13
17-2 × 11-1-F_2-8	8	8	17-2 × 11-1-F_2-22	1	14
17-2 × 11-1-F_2-9	9	4	17-2 × 11-1-F_2-23	0	6
17-2 × 11-1-F_2-10	8	6			

was made in a later year on a portion of the same material that supplied the data in table 3. The observations do not differ significantly for the two seasons. The 23 F_1 plants studied were fertile-pollen plants taken from the reciprocal cross which had the higher proportion of sterile-pollen plants. All 23 F_1 plants produced progenies that showed segregation except 1 in which the 6 individuals were fertile-pollen plants. The range of segregation obtained indicated the presence of genetic differences between these fertile-pollen F_1 plants.

DISCUSSION

Both pollen fertility and pollen sterility have an important function in the improvement of the potato. Fertile-pollen plants are essential for the improvement of the potato through the generally accepted sexual method of breeding, while the sterile-pollen plants, being nonfruitful, will produce, other things being equal, a larger yield of tubers.

Krantz (*8*) suggests developing sterile-pollen and fertile-pollen plants with superior breeding value and then crossing sterile-pollen with fertile-pollen plants to secure nonfruittul improved varieties. The present study indicates how a breeder may proceed in order to produce improved sterile- and fertile-pollen plants. The breeder can, by choosing female parents according to their percentage of stainable pollen, obtain progenies that vary widely or progenies that approach equality in proportion of sterile-pollen to fertile-pollen plants. Female parents with 70 percent or more of stainable pollen produced progenies with a relatively high proportion of fertile-pollen plants, while female parents with little or no stainable pollen produced progenies with a

relatively low proportion of fertile-pollen plants. The female parents having between 10 and 70 percent of stainable pollen produced progenies with sterile-pollen and fertile-pollen plants in proportions that would be suitable for the selection of superior plants of both types. In the 70-percent group, a higher accuracy of estimate of breeding behavior as female parents may be obtained by supplementing th information on stainable pollen with that on breeding behavior, when selfed. Attention should be called to the apparent absence of association between both the percentage of stainable pollen in a plant and its breeding behavior as a female parent with its breeding behavior as a male parent. Significant and consistent differences between plants when used as male parents were observed. Thus the known breeding behavior of a plant when used as a male parent may prove to be a further aid to the breeder in producing the type of progenies in respect to pollen sterility that he desires.

SUMMARY

The problem presented in potato breeding by the anomalous relation of pollen sterility to efficient breeding procedure and to tuber yield was studied.

The breeding material was classified as sterile-pollen and fertile-pollen plants, and the latter were further classified according to their percentage of pollen grains stainable with acetocarmine.

Wide differences were found in the proportion of sterile- to fertile-pollen plants in five sets of reciprocal crosses. Each reciprocal set was made between two parents having significantly different percentages of stainable pollen. In each set the combination low × high percent stainable pollen gave a low proportion and the high × low a high proportion of fertile-pollen plants.

In 5 of nine plants tested the selfed progeny was similar in the proportion of sterile- to fertile-pollen plants to the crossed progeny when the plant was used as a female parent. The significant differences found between the two types of progenies for 2 of the plants indicated that in some combinations the pollen parent influenced the proportion of sterile- to fertile-pollen plants in the progeny.

A study of eight groups of crosses with the progenies within each group having the same female parent showed that progenies with the same female parent but with different male parents may be significantly different in the proportion of sterile- to fertile-pollen plants. This difference was neither related to the percentage of stainable pollen in the male parents nor to their breeding behavior when selfed.

A similar study of seven groups of crosses with the progenies within each group having a common male parent showed that progenies having a common male parent but different female parents may differ widely in the proportion of sterile-pollen to fertile-pollen plants. This difference was associated with the percentage of stainable pollen in the female parent and its breeding behavior when selfed.

Twenty-three fertile-pollen F_1 plants from a cross of low × high percent stainable pollen produced selfed progenies of which all except one segregated for sterile-pollen and fertile-pollen plants. This one produced six plants all of which were fertile-pollen plants.

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STUDIES ON BIOLOGICAL RACES OF THE HESSIAN FLY[1]

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INTRODUCTION

With the increasing interest and progress in breeding wheats resistant to the hessian fly (*Phytophaga destructor* (Say)), the authors feel that the information they have obtained since 1935 on biological races of the fly, i. e., races differing greatly in their ability to infest different varieties of wheat through apparently identical taxonomically, may be of timely value. This information supports the earlier conclusions of Painter (*6*).[2]

ISOLATED GEOGRAPHICAL POPULATIONS OF THE FLY

The hypothesis of biological races of the hessian fly has been offered to explain the contradictory results in fly-resistance tests on wheat varieties in regions of the United States that are separated by physical barriers or distinguished by climatic differences. A striking variability of reaction occurs, for instance, in the variety Dawson, which is resistant in California (*5*) and Kansas (*4*), but susceptible in Illinois and Indiana.[3]

When the testing program on wheat varieties was expanded in 1936 in California, a nursery of 275 American wheat varieties and strains was seeded and exposed to a severe attack of the hessian fly in the field at Birds Landing, Calif. Thirty varieties, including China, Clarkan, Dawson, Dixon, Emerald, Goens, Huston, Java, Marquillo, Marvel, Nabob, Red Rock, Shepherd, Mediterranean, and others, showed high resistance to the fly.

These resistant varieties were retested at La Fayette, Ind., in 1936 in the field, and the complete series of classified wheats (*3*), except for a few omissions, was tested in the greenhouse during the period from 1938 to 1940, inclusive. With the exception of Dixon, Java, Marquillo, and Marvel, the common varieties of wheats were all susceptible to the general population of Indiana hessian flies. Although these results were explainable on the basis of a difference in ability of the California and Indiana populations of the fly to infest the wheats, the possibility that they were due to variation in the wheats themselves in response to environmental differences between the two regions was not eliminated. A small number of resistant and susceptible wheat varieties was therefore tested under uniform environmental conditions in the greenhouse

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[2] Italic numbers in parentheses refer to Literature Cited, p. 153.

[3] Unpublished data on several tests during the period 1920–44.

during 1936, 1939, and 1940 at La Fayette, Ind., flies emerging from flaxseeds collected at Birds Landing, Calif., and at La Fayette being used.

The wheats were infested in the young seedling stage under cages according to a method in use at the La Fayette laboratory (*1*). That there were differences in the reactions of the respective varieties to the flies from the two regions is apparent from the data presented in table 1. The records there shown are in agreement with those of the earlier field tests, and this fact strengthens or confirms the hypothesis that there are distinct regional races of the hessian fly in California and Indiana.

TABLE 1.—*Extent of infestation by hessian flies from California and Indiana in wheat varieties grown in the greenhouse at La Fayette, Ind., 1936 and 1939-40*

Variety of wheat	California flies					Indiana flies				
	1936	1939	1940A	1940B	Average	1936	1939	1940A	1940B	Average
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Dawson	0	11	5	10	6.5	89	89	90	100	92.0
Ill. No. 1 W38-6	16	11	14	10	12.7	5	63	15	25	27.0
Marquillo	.....	39	35	33	35.7	.....	31	35	40	35.3
Wabash	14	31	43	32	30.0	87	84	95	95	90.2
Big Club	80	100	100	95	93.7	100	95	95	100	97.5

HOST-RESTRICTED RACES

Tests reported by Painter (*6*) and Painter, Salmon, and Parker (*7*) showed that there were differences in the capacity of individual flies within a single population to infest different varieties of wheat in the greenhouse. Such findings are highly important and more significant than the occurrence of geographical races of the hessian fly. If a given population in a locality is composed of interbreeding individuals differing with respect to their ability to infest different wheat varieties, the planting of a variety resistant to most but not all individuals in the fly population may result in the natural selection of a local race or population of flies that will be capable of heavily infesting the erstwhile resistant wheat. In view of the importance of obtaining more information on this possibility, efforts to determine whether such a race can be isolated experimentally were made at La Fayette.

The genetic features of the problem resemble those encountered in the study of cereal smut and rust fungi, and a similar technique of investigation is adaptable to their study. Adult hessian flies reared from the few puparia found in resistant wheat were used as the parents of possible host-restricted races. The flies bred and used experimentally during the period 1936–42 did not increase or survive beyond the second generation on resistant wheats. In 1943, however, a selected population which showed a greater ability than that of the general population to mature on Java, Dixon, Marvel, and Ill. No. 1 W38 was obtained in the course of routine tests in the greenhouse from a series of resistant American and foreign wheats including both common and durum varieties. This population was bred and tested in March 1943 and during the period from October 1943 to March

1944. In the tests reported herein this selected population of the fly was compared with the general fly population which had been used continuously for experiments in the greenhouse for 2 years and, because of continuous inbreeding and use on resistant wheats, may have differed from a strictly wild population. It is certain that the general population had within its composition some individuals genetically similar to the ones comprising the selected population, inasmuch as it was the source of the selected population, but logically such individuals would be present in smaller numbers. In all these tests eggs were permitted to be laid on the plants in such numbers as to eliminate the possibility that lack of oviposition on the part of the flies representing either the selected or the general population would account for differences in final infestation.

The first generation of the selected population of the hessian fly was tested on 5 resistant wheats in the greenhouse in March 1943. Four 1-row plantings of young wheat seedlings of each variety, each containing about 20 plants, were exposed to 400 flies of the selected population, and at the same time 4 similar plantings were exposed to 400 flies of the general population. As shown in table 2, the percentage of plants stunted by infestation (plants reacting as susceptible), the percentage of plants infested including those stunted, and the total puparia were significantly greater for the selected population with the exception of those on the highly resistant durum wheat P. I. 94587.

TABLE 2.—*Extent of infestation by hessian flies from selected and general populations in wheat varieties grown in the greenhouse at La Fayette, Ind., March 1943*

Variety	Flies from selected population				Flies from general population			
	Plants examined	Plants infested	Plants stunted [1]	Puparia	Plants examined	Plants infested	Plants stunted [1]	Puparia
	Number	*Percent*	*Percent*	*Number*	*Number*	*Percent*	*Percent*	*Number*
Ill. No. 1 W38-6-11	89	44	24	89	78	5	1	7
IVy	83	34	20	93	83	20	4	50
P. I. 111245-10	80	23	14	36	73	8	7	16
P. I. 56206-8-7	110	7	7	25	109	4	3	5
P. I. 94587	98	0	0	0	76	0	0	0
Michigan Amber (susceptible check)	80	100	100	551	80	100	100	671

[1] Stunted by infestation.

The second generation was reared as stock material in October and November 1943, on the fly-resistant wheats Ill. No. 1 W38, B36162A13-12, and A3848A5-5. The number of individual flies emerging after the storage of material during the summer was too small for tests in this generation. The wheat lines B36162 and A3848 mentioned above are soft, winter-type segregates derived from the backcrossing of Ill. No. 1 W38 with soft red winter wheats in the cooperative project for breeding wheats resistant to the hessian fly at the Purdue University Agricultural Experiment Station.

The third, fourth, fifth, and sixth generations of the selected population of the hessian fly came from rearings through the fly-resistant

wheats B36162A13–12 and A3848A5–5 in the greenhouse during the period December 1943 to March 1944, inclusive. With each successive generation through the resistant wheats, the infestations of B36162A13–12 and A3848A5–5 and of the susceptible check Wabash by the selected and general populations were recorded. In each test 50 female flies selected at random from the respective populations were used to infest the individual plantings, which consisted

FIGURE 1.—Wheat strains exposed to selected and general populations of the hessian fly: Rows *a* to *c* exposed to selected population of the fly, and rows *d* to *f* to the general population. Rows *a* and *f*, Wabash (susceptible); rows *b* and *e*, B36162A13–12 (resistant); and *c* and *d*, A3848A5–5 (resistant).

of approximately 45 plants of each of the three wheats. A summary of all tests is given in table 3.

TABLE 3.—*Extent of infestation by selected and general populations of hessian flies in B36162A13–12, A3848A5–5, and Wabash wheats grown in the greenhouse at La Fayette, Ind., December 1943 to March 1944*

Variety	Total tests	Flies from selected population		Flies from general population	
		Infested plants	Stunted plants [1]	Infested plants	Stunted plants [1]
	Number	*Percent*	*Percent*	*Percent*	*Percent*
B36162A13–12	33	73.5	62.6	43 3	33.5
A3848A5–5	32	67.6	58 8	36.6	27 5
Wabash	33	100	100	100	100

[1] Stunted by infestation.

Table 3 shows that the percentage of susceptible or stunted plants in the resistant varieties infested with the selected population of the

fly was approximately double that of plants infested with flies from the general population. There were highly significant visual differences in the reactions of the two populations (fig. 1) which were apparent in most tests.

Infestations of 80 percent or more of the plants by the selected population were not uncommon in the tests, but infestations varied greatly among the individual tests. This variation indicates that the selected population was not homozygous after six generations of screening through the resistant wheats. The results appear to demonstrate quite conclusively, however, that a strain or race of the hessian fly capable of maturing successfully in and seriously injuring wheats that are highly resistant to the general fly population can be segregated from that population.

TABLE 4.—*Extent of infestation by hessian flies from the fourth-generation selected population in wheat varieties grown in the greenhouse at La Fayette, Ind., March 1944*

Variety [1]	Plants examined	Plants infested	Plants stunted [2]
Common wheat:	*Number*	*Percent*	*Percent*
Wabash, check	249	100	100
B36162A13-12, check	202	98	96
Alberta Early, C. I. 10025-2	28	100	100
Centenario, Ks 38 F N 4002	27	96	96
Dixon, C. I. 6049	30	93	93
Greek 10, P. I. 116227	30	100	100
IVcl, Ks. 36 R N 3579	25	96	96
IVy, Ks. 36 R N. 3580	24	88	88
Java, C. I. 10051	35	97	97
Marquillo, C I 6887	27	37	37
Marvel, C I. 8876	26	100	100
Portugez, P. I 56204-7	30	13	13
Ribeiro, P. I. 56206-8	27	0	0
Triunfo, P. I 104138	22	100	100
Unnamed, P. I. 94549-6	29	14	14
Unnamed, P. I. 94571-14	32	9	6
Unnamed, P. I. 111245-10	32	100	100
Durum wheat:			
Tremez rijo, P I. 56257-1	25	0	0
Unnamed, P. I. 94587	42	0	0
Emmer:			
Yaroslav, C. I. 1562	62	0	0

[1] C. I. after varietal name refers to accession number of the Division of Cereal Crops and Diseases and P. I. to accession number of the Division of Plant Exploration and Introduction, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, and Ks. to the accession number of the Kansas Agricultural Experiment Station.

[2] Stunted by infestation.

Seventy-eight wheat varieties and strains resistant to the general population of the hessian fly (*2*) were tested with the selected population of the fourth generation in March 1944. Many adult flies emerging from the stocks of the selected population were permitted to oviposit heavily on the wheat seedlings, thereby increasing the chances of infestation by individuals having the ability to mature in them. The percentage of stunted or susceptible plants resulting in most of the common varieties approached that of the susceptible check, but the common wheat variety Ribeiro, the highly resistant durums, and the emmer variety Yaroslav were uninfested. A partial list of the varieties and their reaction to the selected population is

given in table 4 to show the variation in infestation among those included in this test. In this series a check of the plants of B36162A13–12 showed 41 percent stunted by the general population of the fly in contrast to the 96 percent for the selected population, which indicated that the expression of susceptibility in the resistant wheats was not due to adverse environmental conditions in the greenhouse at the time of the tests. The probability of further segregation of specialized strains of flies through host restriction of populations is indicated by the differences in infestations occurring in such common varieties as Marquillo C. I. 6887, Ribeiro P. I. 56206–8, and unnamed P. I. 94549–6.

The results of the several tests reported in this paper indicate that a fully effective breeding program for the development of fly-resistant commercial hybrids or varieties of wheat must provide for the possible appearance of biological races of the hessian fly within regions as well as for their present known occurrence in different regions. This involves not only the study of genetically different factors for hessian fly resistance in wheat varieties, but also a study of the genetic diversity of fly populations.

SUMMARY

The tests at La Fayette, Ind., since 1935 have substantiated the existence of different biological races of the hessian fly in California and Indiana indicated in previous studies by the writers and others. The characteristics of the two populations as noted in field trials in the two regions were maintained when tested on resistant and susceptible wheats in the greenhouse at La Fayette, Ind. Varieties such as Dawson and Wabash, which were resistant to the California populations of the fly, were susceptible to the Indiana population.

In the studies on host-restricted races of the hessian fly, a population was bred from the general population at La Fayette which was capable of heavily infesting many resistant common wheats, such as Dixon, Java, and Marvel. This selected population was bred and tested for six generations on resistant wheats, including the winter lines derived from crosses with Ill. No. 1 W38. The percentage of plants stunted or susceptible in the resistant varieties was doubled after breeding the selected population through the third to sixth generations, but it did not attain the 100 percent injury of susceptible wheats.

The selected population of the hessian fly was cultured for more extensive tests on resistant wheats, and the differences in infestations obtained in varieties such as Marquillo, Ribeiro, and other varieties indicated the probability of further segregation of the selected population. Several durum wheats which appeared to be immune to the general population of the hessian fly were uninfested by the selected population.

The results indicate that a fully effective program for breeding resistance to the hessian fly must provide for the possible appearance of biological races of the fly within regions as well as for their known presence in different regions, through a study of the genetic diversity of fly populations as well as a study of the genetically different factors for hessian fly resistance in different varieties of wheat.

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VALUE OF SOYBEAN MEAL PREPARED FROM FROSTED-FIELD-DAMAGED SOYBEANS FOR GROWING-FATTENING SWINE [1]

By B. W. FAIRBANKS, formerly *chief in swine husbandry;* J. L. KRIDER, *associate chief in swine husbandry;* DAMON CATRON, formerly *associate in animal husbandry;* and W. E. CARROLL, *chief in swine husbandry and head, Department of Animal Husbandry, Illinois Agricultural Experiment Station* [2]

INTRODUCTION

During the fall of 1942 large areas of soybeans in the central part of the Corn Belt were frosted before maturity, and the quality of the beans was further reduced by unfavorable weather which postponed harvest until late winter or early spring. Such beans are referred to in this paper as frosted-field-damaged soybeans.

Soybean meal was prepared by the expeller process from a sample of these frosted-field-damaged soybeans for comparison with expeller soybean meal prepared from sound mature beans of the 1942 crop. The damaged soybeans, which were harvested in 1943, are thus described by T. H. Allwein: [3]

> These beans were just straight country run beans, which were harvested from the fields during March. These beans were of mixed varieties, which originated in this locality [Gibson City, Ill.]. All of these beans were sample grade beans and averaged about 40 percent damage and 48 pounds test weight. There also was considerable dockage, on an average of about 3 percent. This meal was produced by our regular expeller process. . . .

METHODS

In order to obtain quantitative results the paired-feeding method for equal gains was used. One pig of each pair was fed the check ration which contained normal soybean meal, while its pair mate was fed the mixture which contained soybean meal prepared from the frosted-field-damaged soybeans.

Sixteen Poland China pigs which had been on rye and alfalfa pastures during the spring and early summer were paired on the basis of weight, litter, sex, type, condition, and probable outcome. All pigs were thrifty.

The basal mixture used consisted of ground yellow corn, soybean meal, alfalfa meal, and a mineral mixture. The proportion of corn

[1] Received for publication June 25, 1945.

[2] The writers gratefully acknowledge the assistance of R. H. McDade, chief swine herdsman.

[3] Personal correspondence. The damaged soybean meal was prepared and donated to the University of Illinois by the Central Soya Co., Inc., Gibson City, Ill.

and soybean meal in the mixture was varied in order to change the percentage of protein in the ration as the pigs increased in weight. The percentages fed are given in table 1. The alfalfa meal was fed at the 10-percent level during the test period. The generous feeding of alfalfa meal was to insure against a possible vitamin deficiency in the all-vegetable ration, as discussed by Krider, Fairbanks, and Carroll.[4]

TABLE 1.—*Variations in the proportion of the ingredients of the rations fed to growing swine at different live weights*

Feeds	Proportion in which feeds were mixed for pigs weighing—					
	75 pounds or less		75 to 150 pounds		Over 150 pounds	
	Check ration	Test ration	Check ration	Test ration	Check ration	Test ration
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Ground yellow corn	53.0	53.5	65.0	65.5	71.0	71.0
Normal soybean meal	34.5		22.5		16.5	
Damaged soybean meal		34.0		22.0		16.5
Alfalfa meal	10.0	10.0	10.0	10.0	10.0	10.0
Ground limestone	.5	.5	.5	.5	.5	.5
Steamed bonemeal	1.5	1.5	1.5	1.5	1.5	1.5
Iodized salt	.5	.5	.5	.5	.5	.5
Total	100.0	100.0	100.0	100.0	100.0	100.0
Crude protein (percent)	21.0		17.0		15.0	

The yellow corn, which graded No. 2, was estimated to contain 9.0 percent crude protein. Analysis of the alfalfa meal showed 14.6 percent crude protein. The chemical composition of the soybean meals is given in table 2.

In preparing the feed mixtures, the slightly higher protein content of the damaged soybean meal was taken into consideration by equalizing the total percentage of crude protein in the mixtures for the pigs in each weight-group interval. The percentages of crude protein fed are given in table 1.

The pigs were fed twice daily in individual feeding crates. The feed allowances were weighed to one-tenth of a pound and fed in a metal trough with which each crate was equipped. A small amount of water was poured on the feed to prevent waste. The pigs were closely observed in an attempt to keep feed consumption up to the limit of the gains of the slower gaining pig of each pair. Feed refusals were noted. Individual weights of all pigs were taken at weekly intervals and the feed allowances for the following week were adjusted in accordance with the gains made during the previous week. This method was considered satisfactory, as reported previously by Krider, Fairbanks, and Carroll.[4]

[4] KRIDER, J. L., FAIRBANKS, B. W., and CARROLL, W. E. VALUE OF SOYBEAN MEAL PREPARED FROM DAMAGED (BIN-BURNED) SOYBEANS AS A FEED FOR GROWING SWINE. Jour. Agr. Res. 69: 383–387. 1944.

TABLE 2.—*Chemical composition of the soybean meals on the fresh basis*

Feeds	Dry substance	Crude protein	Ether extract	Ash	Crude fiber	Nitrogen-free extract
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Normal soybean meal	88 76	42 75	4. 65	5. 14	4 58	31. 64
Damaged soybean meal	88 41	43. 25	4 79	5. 39	4. 45	30. 53

The eight pairs of pigs were kept on concrete floors, in one group, and had access to an outside concrete runway.

The experiment began on July 13, 1943, and continued until both pigs of each pair had attained a final weight of approximately 200 pounds.

RESULTS AND DISCUSSION

The results of the experiment are summarized in table 3. The table shows that in five of the eight pairs the check pig required more feed per pound of gain, while in three pairs the test pig required more. These results on economy of gain are too nearly a chance distribution to indicate any significant differences in the feeding value of the two mixtures. This conclusion was verified by statistical treatment of the data by Students' method with argument t for paired differences as described by Snedecor.[5]

The record of feed refusals indicated that the rations were equally palatable, and the χ^2 test showed that the difference in feed refusals was largely a chance deviation.

To obtain ideal results in paired feeding for equal gains, the pigs of each pair should make the same weekly gain in live weight. However, this rarely happens. With the 8 pairs of pigs used in these experiments, there was a total of 177 weekly comparisons between the gains of pair mates. Of the 177 comparisons, only 17 showed identical gains, while in 82 the check pigs gained slightly more and in 78 the test pigs gained more. The deviation of 2 from the expected, assuming a chance distribution, is not significant, which indicates that the method of feeding for equal gains used in the present work was quite successful.

The average daily gains are probably smaller than the feed mixtures are capable of supporting under conditions of unrestricted feeding, but this is to be expected when either gain or feed intake is controlled in paired feeding.

It may be observed in table 3 that the average daily feed was greater for the check pig in 5 pairs and greater for the test pig in 3 pairs. From an analysis of the 177 weekly comparisons of the feed intake of pair mates, it was found that slightly more of the check ration was required to produce the same gains as the ration which contained damaged soybean meal. In 19 of these comparisons, pair mates consumed the same amount of concentrates, in 98 the check pigs consumed more, and in 60 the test pigs consumed more. The χ^2 test indicates that the deviation of 19 from the ideal of a chance

[5] SNEDECOR, G. W. STATISTICAL METHODS APPLIED TO EXPERIMENTS IN AGRICULTURE AND BIOLOGY. Ed. 3, 422 pp., illus. 1940. Ames, Iowa.

TABLE 3.—*Weights, gains, and feed consumption of 8 pairs of pigs, one of each pair being on the check ration and the other on the damaged soybean-meal (test) ration*

Item	Pair 1		Pair 2		Pair 3		Pair 4		Pair 5		Pair 6		Pair 7		Pair 8		Average	
	Check pig	Test pig	Check pig	Test pig	Check pig	Test pig	Check pig	Test pig	Check pig	Test pig	Check pig	Test pig	Check pig	Test pig	Check pig	Test pig	Check pig	Test pig
Final weight.....pounds..	205	200	205	208	214	212	197	202	198	198	202	200	207	201	205	202	204.1	202 9
Initial weight.....do....	66	60	50	58	62	62	49	48	45	47	70	72	74	65	60	59	59.5	58.9
Total gain.....do....	139	140	155	150	152	150	148	154	153	151	132	128	133	136	145	143	144.6	144.0
Period on test.....days..	147	147	147	147	147	147	182	182	154	154	154	154	147	147	161	161	155	155
Average daily gain.....pounds..	.95	.95	1 05	1.02	1.03	1.02	81	85	.99	.98	.86	.83	.90	.92	.90	.89	.94	.94
Total feed eaten.....do....	524.4	493.4	530 9	490 6	429 9	525 3	579.8	537 7	504 5	485 3	466.6	487.2	537.4	502.1	500.5	505.8	517.1	503.4
Average ration.....do....	3.57	3.36	3.61	3 34	3 35	3.57	3.19	2.95	3 28	3.15	3.03	3.16	3.66	3.42	3 11	3 14	3.35	3.26
Feed consumed per pound of gain...do..	3.77	3 52	3 43	3 27	3.24	3.50	3 92	3 49	3.30	3.21	3.53	3 81	4 04	3.69	3 45	3.54	3.58	3 50

distribution was not due to chance alone, and that some other factor or factors were probably operating. The χ^2 value of 9.14 is significant. The authors believe that the values for feed per 100 pounds of gain are more important criteria than the comparison of weekly feed intake and hence that little or no significance should be attached to the comparisons of the weekly feed intakes.

Within the limits of error of the method, the data indicate that the two meals have practically the same energy value. Since the levels at which protein was fed were not border line but were considered optimum, it cannot be concluded that protein in the meal from the frosted-field-damaged soybean is as good for promoting growth as that in the meal from the normal soybean. At the protein levels fed, however, the two meals were equally efficient in supplementing corn, alfalfa meal, and minerals for growing-fattening pigs fed in dry lot under the conditions of this experiment.

The inclusion of 10 percent of alfalfa meal in the rations may possibly have obscured differences in the heat-labile constituents, such as some of the vitamins, contained in the two soybean meals. The purpose of adding the alfalfa meal was to prevent the vitamin deficiencies which occur when a ration containing corn, soybean meal, and minerals is fed to pigs in dry lot.

SUMMARY AND CONCLUSIONS

In the central Corn Belt large areas of soybeans of the 1942 crop were frosted before maturity and were further damaged by unfavorable weather which prevented harvest until the spring of 1943. Some of these frosted-field-damaged soybeans were described as Sample Grade mixed soybeans, 40 percent damaged, with a test weight of 48 pounds per bushel. Soybean meal prepared by the expeller process from these damaged soybeans was compared with soybean meal, prepared by the same method, from sound soybeans in feeding experiments with growing-fattening pigs.

The method of paired feeding for equal gains was used in this test, which involved eight pairs of pigs fed in dry lot. The soybean meals supplemented feed mixtures composed of ground yellow corn, alfalfa meal, steamed bonemeal, ground limestone, and iodized salt. In three pairs of pigs, the checkmates made more economical gains than the pigs fed the soybean meal from frosted-field-damaged beans, while in five pairs the economy of gains favored the latter pigs. These results were studied statistically and the differences were found to be statistically insignificant.

It is concluded (1) that the energy value of the two meals is the same for growing-fattening pigs, and (2) also at the protein levels fed, the two meals were equally efficient in supplementing corn, alfalfa meal, and minerals

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GROWTH RATES OF HOST AND PATHOGEN AS FACTORS DETERMINING THE SEVERITY OF PREEMERGENCE DAMPING-OFF [1]

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INTRODUCTION

Severity of preemergence damping-off is determined by several factors, among which mass of inoculum, host susceptibility, temperature, and soil moisture are usually the most important. Many workers have studied the effect of temperature upon seedling diseases. Two outstanding papers are those by Dickson (*3*),[2] who demonstrated that *Gibberella saubinetii* (Mont.) Sacc. produced severe infection upon corn at low temperatures and upon wheat at high temperatures, and by Jones, Johnson, and Dickson (*9*), who summarized the results of a number of temperature studies. The latter writers raised the question whether the dominant influence of temperature was through the pathogen or through the host. They concluded that with *G. saubinetii* the effect must be through the host. The relative susceptibility of the host at different temperatures was explained by Dickson and Holbert (*4*) as due to chemical differences within the host. At about the time that Dickson (*3*) was making his experiments with *G. saubinetii* Richards (*20*) tested the pathogenicity of *Rhizoctonia solani* Kühn upon several hosts and from these investigations Jones, Johnson, and Dickson (*9, p. 59*) concluded that the "relation of temperature to parasitism with *Rhizoctonia* is a fixed character of the fungus" and that "neither the nature of the host nor its normal temperature relations materially influence the temperature range for the parasitic action of this fungus."

In the present studies the pathogenicity of several damping-off fungi has been tested on different hosts in constant-temperature chambers with uniform soil moisture. Upon the same host several specific organisms show different temperature ranges for infection. Likewise a single organism may have different optimum temperature ranges for infection of different hosts. Usually the relative severity of infection at different temperatures did not correspond closely to the growth rate of either the host or the pathogen. As a rule, however, the percent of seedlings emerging from infested soil at different temperatures agreed closely with the ratio between the coefficient of velocity (*11*) of emergence and the growth rate of the organism at the same temperatures (*12, 13*). Since this relation held for several combinations of hosts and pathogens, conceivably it might have a general

[1] Received for publication October 4, 1946.

[2] Italic numbers in parentheses refer to Literature Cited, p. 178.

application. Utilization of this principle would aid in the selection of planting periods that permit the seedlings to escape infection from some seedling pathogens and to avoid severe infection from others.

PROCEDURE

All tests were conducted in a series of eight thermostatically controlled temperature chambers at 4° to 35° C. Temperature variations were less than 1° in all chambers except the 4° one, where readings as high as 6° or 7° were sometimes recorded during the long periods required for germination.

DETERMINATION OF SEEDLING EMERGENCE RATES

For studying seedling emergence Yolo fine sandy loam first was pasteurized at 80° to 90° (*21*) and then was moistened to field capacity [3] (16 percent) with a spray of distilled water while the soil revolved in an electrically driven cement mixer. Plantings were made in the manner described by Doneen and MacGillivray (*5*). Ten No. 2 tin cans with friction tops and each containing 300 gm. of this soil were placed in each temperature chamber along with a reserve of soil for covering the seeds. The following day, after the soil in each chamber had reached a constant temperature, 10 seeds of the host to be tested were placed in each can and covered with 100 gm. of soil from the same chamber. The cans were then topped and immediately replaced in the constant-temperature chamber. Each day the lids were removed so that germination might be observed and so that gases might not accumulate and inhibit germination.

The emerged seedlings were counted daily during the emergence period and the coefficient of velocity of emergence at each temperature was calculated by the Kotowski (*11*) formula:

$$\frac{\text{Total emergence at end of period}}{\text{Sum of (each daily emergence increase} \times \text{days since planting)}} \times 100 = \text{C. V. E.}$$

The rate of emergence can also be expressed by the mean emergence period (*14*), calculated in the same way as the coefficient of velocity except that the sum of the products is divided by the total emergence at the end of the trial. Or the coefficient of velocity divided into 100 is equal to the mean emergence period expressed in days. For example, a coefficient of velocity of 4 indicates a mean emergence period of 25 days, whereas one of 20 represents a mean emergence period of 5 days. The results in table 1 show that, relatively speaking, spinach is favored by low or moderate temperatures, whereas watermelons emerge rapidly only at high temperatures, a difference that is well known. Garden peas, wheat, and sugar beets emerge fairly rapidly at low temperatures, but are intermediate between the low- and high-temperature crops mentioned above.

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[3] The writer is indebted to Dr. L. D. Doneen, of the Irrigation Division, University of California, Davis, for adjusting the soil moisture and for making all moisture determinations.

TABLE 1.—*Relation of temperature to emergence rate of seeds planted in pasteurized soil*

Temperature (° C.)	Coefficient of velocity of emergence [1]				
	Spinach	Wheat	Garden peas	Sugar beets	Watermelons
4	4.2	2.6	2.4	1.6	0
8	7.1	5.1	4.6	4.6	0
12	10.3	10.1	8.8	8.4	0
16	15.7	13.2	10.5	10.8	3.6
20	17.6	15.2	12.3	16.1	8.5
25	19.5	20.9	16.8	23.4	21.1
30	15.4	22.9	15.1	23.8	28.7
35	None	None	None	21.9	33.0

[1] See definition, p. 162.

DETERMINATION OF FUNGUS GROWTH RATES

Some of the difficulties involved in measuring fungus growth rates have been reported by Fawcett (*6*). The time factor is important but it is difficult to secure comparable rates without selecting more or less arbitrary periods for the different temperatures. Measurements on potato-dextrose agar were secured by placing a 2-mm. disk of agar, from near the periphery of a young fungus colony, as inoculum in the center of preincubated agar plates. At each temperature, radial growth was measured at intervals of 24 hours or less until the colony approached the edge of the petri dish, and the average growth in millimeters per 24-hour period was calculated. Near the optimum temperature, the maximum growth permitted by the dish was sometimes reached within 48 hours, whereas at low temperatures several weeks were required by some organisms.

TABLE 2.—*Growth rates of 4 damping-off pathogens*

Temperature (°C.)	*Pythium ultimum* on—		*Rhizoctonia solani* on solid medium	*Aphanomyces cochlioides* on solid medium	*Phoma betae* on solid medium
	Solid medium	Liquid medium			
	Millimeters per 24 hours	*Milligrams per 24 hours*	*Millimeters per 24 hours*	*Millimeters per 24 hours*	*Millimeters per 24 hours*
4	0 8	1.0	0	0	0 7
8	4.1	3.6	.1	.6	1.3
12	10.9	12.2	3.1	2.1	2.4
16	17.2	17.2	6.9	3.9	3.0
20	22.4	22.2	13 4	5.4	4.3
25	29.1	19.4	19.0	7.6	4.7
30	27.5	15.6	19.6	8.8	3.6
35	6.8	8.0	9.7	4.6	.4
40	0	0	1.0	----------	----------

As table 2 shows, the optimum temperature range for *Pythium ultimum* Trow appeared to fall between 25° and 30° C. This corresponds to the results obtained by Middleton (*17*), who found the highest growth rate at 28°, but it is considerably lower than the optimum of 32° reported by Harter and Whitney (*7*) for an isolate of this species from sweetpotato.

Since the mycelial growth of *Pythium ultimum* was less dense at 30° and 35° C. than at 20° or 25°, the growth rate was also determined by dry-weight yield in a liquid medium. Flasks of potato-dextrose broth were inoculated in triplicate at each temperature. Again the

length of the incubation period had to be adjusted according to the rapidity of growth. At the end of the incubation period the fungus colony was separated from the medium in a filter and then washed and dried. The growth rate was measured by the average weight of the dried colony in milligrams per 24 hours of incubation. As is shown by the results in table 2, the growth rates of *P. ultimum* on solid and in liquid medium were similar at temperatures of 4° to 20°, but growth at 25° and 30° was considerably less in the liquid medium. Most writers agree that measurement of fungus mass is more reliable than radial growth; and the growth rates in liquid medium are used in all comparisons with *P. ultimum*. Since, however, other fungi such as *Rhizoctonia solani*, *Phoma betae* Frank, and *Aphanomyces cochlioides* Drechs. did not show the same difference in density of colony, only the radial growth rates are used in this paper.

EFFECT OF TEMPERATURE ON PREEMERGENCE DAMPING-OFF

Infested soils were prepared by spraying a suspension of fungus mycelium into the pasteurized soil at the time the moisture content was adjusted. Young colonies of the fungus on agar media were suspended in distilled water by mixing with a Waring blender. With coenocytic fungi such as *Pythium ultimum* and *Aphanomyces cochlioides* excessive mixing in the blender resulted in some loss of viability. The degree of infestation of the soil was governed by the amount of fungus inoculum added and by the time of incubation before planting. In each test the identity of the casual organism was confirmed by pure culture isolation from infected seedlings or by microscopic examination of infected seedlings placed in water culture.

PYTHIUM INFECTION OF SPINACH

To determine the severity of preemergence damping-off for each combination of host and pathogen, germination trials were conducted in pasteurized soil and in soil infested with a specific organism. For example, Prickly Winter spinach seed planted in pasteurized soil at various temperatures germinated at rates indicated by the coefficient of velocity shown in table 3. The percentage of seedlings that

TABLE 3.—*Relation of growth rates of spinach and Pythium ultimum to emergence in infested soil*

Temperature (° C.)	Emergence of spinach, coefficient of velocity [1]	Growth rate of *P. ultimum*	Ratio of growth rates,[2] host to pathogen	Emergence in—	
				Pythium soil	Pasteurized soil
		Milligrams per day		*Percent*	*Percent*
4	4.2	1.0	4.20	95	95
8	7.1	3.6	1.97	12	96
12	10.3	12.2	.84	1	96
16	15.7	17.2	.91	0	95
20	17.6	22.2	.79	5	97
25	19.5	19.4	1.00	16	96
30	15.4	15.6	.99	13	23
35	0	8.0	----------	0	0

[1] See definition, above.

[2] Ratio = $\frac{\text{Emergence rate of spinach (coefficient of velocity).}}{\text{Growth rate of } P.\ ultimum \text{ (milligrams per day)}}$

emerged at each temperature indicates that in the absence of pathogenic organisms spinach germinated about equally well between 4° and 25° C., but very poorly at 30° or above.

In *Pythium*-infested soil, however, preemergence damping-off was severe at all temperatures above 4° C., but especially so between 12° and 20° (table 3). The ratio of growth rate of the host to that of the pathogen (column 4), bears a close relation to the emergence of spinach in *Pythium*-infested soil (fig. 1), with no preemergence infection at 4° where the ratio is above 4.0 but with severe infection where the ratio drops to 1.0 or below.

RHIZOCTONIA INFECTION OF SPINACH

The ratio of the growth rate of spinach (table 4) to that of *Rhizoctonia solani* shows that at 4° and 8° C. the host grew relatively faster than the fungus. At these temperatures there was no evidence of

TABLE 4.—*Relation of growth rates of spinach seedlings and Rhizoctonia solani to emergence in infested soil*

Temperature (° C.)	Emergence of spinach, coefficient of velocity	Growth rate of *R. solani*	Ratio of growth rates,[1] host to pathogen	Emergence of—			
				Seed lot A in—		Seed lot B in—	
				Rhizoctonia soil	Pasteurized soil	Rhizoctonia soil	Pasteurized soil
		Millimeters per day		*Percent*	*Percent*	*Percent*	*Percent*
4	4.2	0	∞	86	73	(2)	95
8	7.1	.1	71.00	81	77	(2)	96
12	10 3	3 1	3.32	65	81	93	96
16	15.7	6.9	2.28	35	77	82	95
20	17.6	13.4	1.31	1	75	34	97
25	19 5	19.0	1.03	0	77	1	96
30	15.4	19.7	.78	0	11	0	23
35	0	9.7	- - -	0	0	0	0

[1] Ratio = $\frac{\text{Emergence rate of spinach (coefficient of velocity).}}{\text{Growth rate of } \textit{R. solani} \text{ (millimeters per day)}}$

[2] Not tested.

preemergence damping-off. As the temperature increased, the ratio of host growth rate to pathogen growth rate decreased, and a corresponding increase in the severity of infection took place with both seed lots A and B (fig. 2,*A* and *B*). Infection was less severe, however, upon seed lot B with high germination than upon seed lot A which showed lower viability.

Kotowski (*11*) found that the germination of spinach seed decreased with each increase in temperature from 5° to 30° C. In the present trials there were no differences in the percent of emergence of spinach seed planted in pasteurized soil between 4° and 25°; although emergence was reduced considerably at 30°. Since Kotowski's results resemble those observed in infested soil, perhaps his sand medium contained a mild infestation of damping-off pathogens, such as *Rhizoctonia solani*.

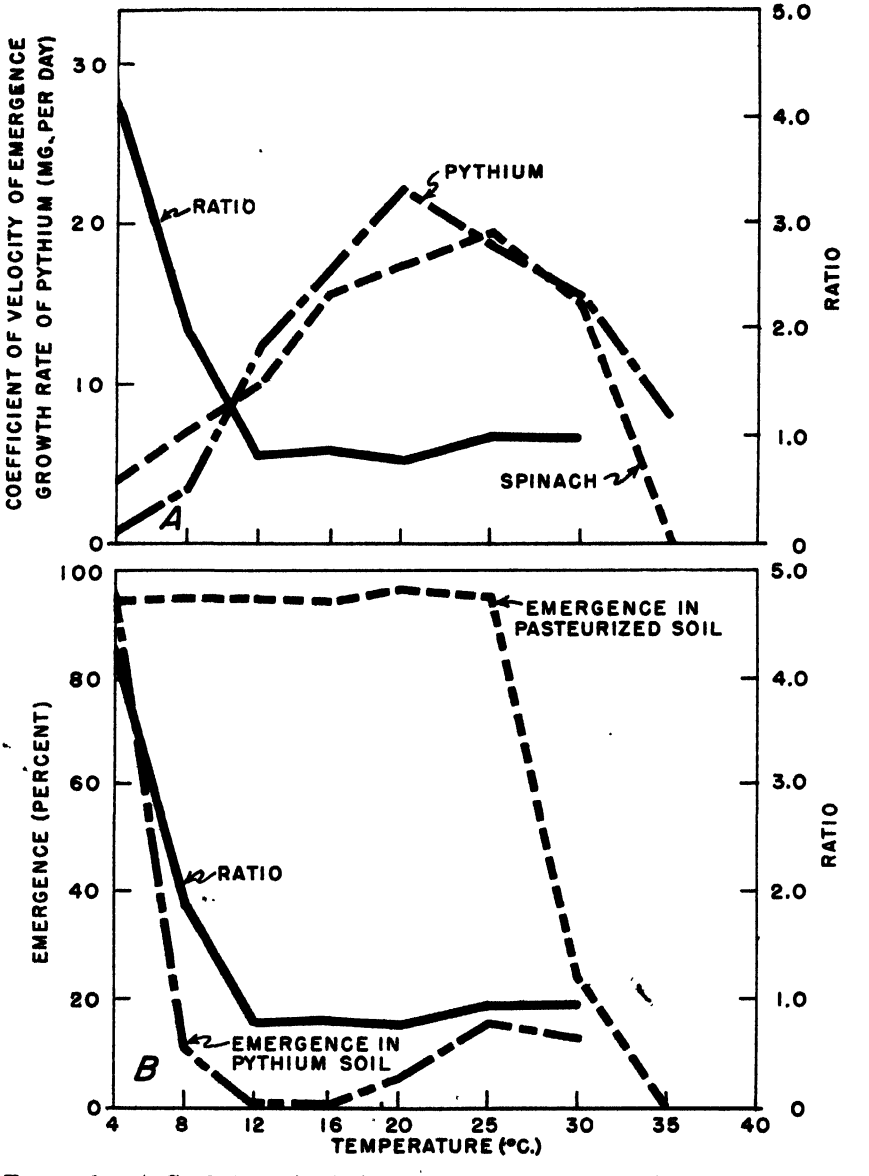

FIGURE 1.—*A*, Coefficient of velocity of emergence of spinach in pasteurized soil, growth rate of *Pythium ultimum* in liquid medium, and ratio of emergence rates of spinach to growth rate of *Pythium; B*, percent emergence of spinach in pasteurized soil and in soil infested by *P. ultimum* compared with curve representing ratio of growth rates of host and pathogen reproduced from figure *1,A*. Spinach escaped infection by *P. ultimum* at 4° C., where the host grew relatively faster than the pathogen, and was most severely infected between 12° and 20°, where the fungus grew relatively faster than the host.

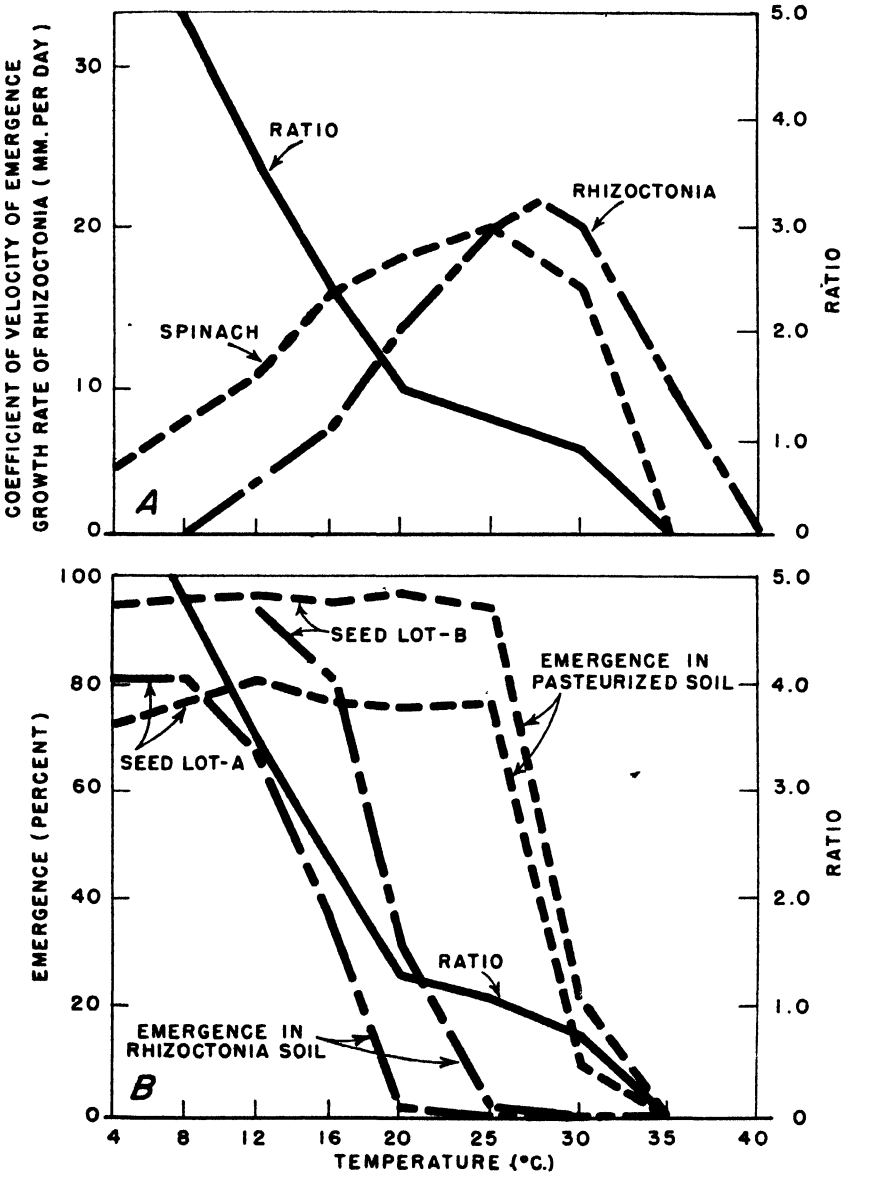

FIGURE 2.—*A*, Coefficient of velocity of emergence of spinach at temperatures of 4° to 40° C., growth rate of *Rhizoctonia solani* on agar, and ratio of emergence rate of spinach to growth rate of *Rhizoctonia*. The low temperatures were more favorable for the host and the high temperatures for the pathogen. *B*, Percent emergence of spinach in pasteurized and in *Rhizoctonia*-infested soil compared with curve representing ratio of growth rates of host and pathogen reproduced from figure 2,*A*.

PYTHIUM INFECTION OF GARDEN PEAS

Garden peas (variety Laxton's Progress) germinate well in moist pasteurized soil at 4° to 30° C., but no emergence was obtained at 35° (table 5). Although peas emerge slowly at 4° and 8°, their growth

TABLE 5.—*Relation of growth rates of peas and Pythium ultimum to emergence in infested soil*

Temperature (° C.)	Emergence of peas, coefficient of velocity	Growth rate of *P. ultimum* milligrams per day	Ratio of growth rates,[1] host to pathogen	Emergence in—	
				Pythium soil	Pasteurized soil
				Percent	*Percent*
4	2.4	1.0	2.40	66	89
8	4.6	3.6	1.28	18	90
12	8.8	12.2	.72	2	98
16	10.5	17.2	.61	0	93
20	12.3	22.2	.55	0	93
25	16.8	19.4	.86	2	94
30	15.1	15.6	.97	32	86
35	0	8.0	——	0	0

[1] Ratio = $\frac{\text{Emergence rate of peas (coefficient of velocity)}}{\text{Growth rate of } P.\ ultimum \text{ (milligrams per day)}}$.

rate is relatively faster than that of *Pythium* at these temperatures (fig. 3, *A*) and infection was less severe than at higher temperatures. Seed decay and preemergence infection were most severe between 12° and 25° (fig. 3, *B*). In this trial the inoculum was so heavy that practically all seedlings were destroyed at these temperatures and differences between the temperatures were obscured.

The occurrence of less infection upon peas at low than at intermediate temperatures confirms Reinking's (*19, p. 41*) conclusion that "peas should be planted as early as possible in order to take advantage of the cooler soil temperatures that are unfavorable to fungous development." McNew (*16*) stated that seed decay was more severe in cool soils (15° to 20° C.) than in warmer ones (29° to 32°). His cool soils fall, however, within the optimum range for infection as shown in table 5.

In all comparisons of temperature effects, soil moisture must be maintained at similar levels, since, as shown by Jones (*8*), soil moisture is perhaps more important than temperature in determining the severity of pea seed decay.

PYTHIUM INFECTION OF SUGAR BEET

Sugar beet seedlings develop faster than *Pythium ultimum* at both high and low temperatures (table 6), but at intermediate temperatures the pathogen develops faster.

In the absence of soil-borne organisms, the greatest number of sugar-beet seedlings was produced at 12° to 30° C., somewhat fewer at 4° and 8°. A temperature of 35°, however, not only reduced the number but resulted in the production of weak seedlings. In *Pythium*-infested soil, preemergence damping-off was most severe at 12° to 20°, where the ratio of the growth rate of the host (table 6) to that of the pathogen was the lowest.

TABLE 6.—*Relation of growth rates of sugar beets and Pythium ultimum to emergence in infested soil*

Temperature (° C.)	Emergence of sugar beets, coefficient of velocity	Growth rate of *P. ultimum*	Ratio of growth rates,[1] host to pathogen	Emergence of seedlings per 100 seed balls in—			
				Pythium soil			Pasteurized soil
				Trial A	Trial B	Trial C	
		Milligrams per day		*No.*	*No.*	*No.*	*No.*
4	1.6	1.0	1.60	54	70	46	114
8	4.6	3.6	1.28	32	18	28	145
12	8.4	12.2	.69	1	0	15	186
16	10.8	17.2	.63	0	0	15	189
20	16.1	22.2	.72	0	0	12	193
25	23.4	19.4	1.21	5	2	38	209
30	23.8	15.6	1.52	49	30	132	192
35	21.9	8.0	2.74	109	141	90	75

[1] Ratio = $\frac{\text{Emergence of beets (coefficient of velocity)}}{\text{Growth rate of } P.\ ultimum \text{ (milligrams per day)}}$.

Since the zone of severe infection coincides with the most favorable temperatures for germination of sugar beets (fig. 4, *A*) and since infection may be fairly severe throughout the normal range for germination (8° to 30° C.) (fig. 4, *B*), there is little possibility of eliminating *Pythium* infection by altering the planting date as long as soil moisture conditions remain favorable for the pathogen.

RHIZOCTONIA INFECTION OF SUGAR BEET

When the growth rates of sugar beets and *Rhizoctonia solani* (table 7) are compared, low temperatures seem to be much more favorable to the host than to the pathogen (fig. 5, *A*). In soil of moderate infestation (trial A), preemergence damping-off was most severe at 20° to 30° C., corresponding to the lowest ratios of the emergence rate

TABLE 7.—*Relation of growth rates of sugar beets and Rhizoctonia solani to emergence in infested soil*

Temporary (°C.)	Emergence of sugar beets, coefficient of velocity	Growth rate of *R. solani*	Ratio of growth rates [1] host to pathogen	Emergence of—		
				Seedlings per 100 seed balls in—		
				Rhizoctonia soil		Pasteurized soil
				Trial A [2]	Trial B [3]	
		Millimeters per day		*Number*	*Number*	*Number*
4	1.6	0	∞		116	114
8	4.6	.1	46.00	122	143	145
12	8.4	3.1	2.71	199	185	186
16	10.8	6.9	1.56	128	3	189
20	16.1	13.4	1.20	83	0	193
25	23.4	19.0	1.23	23	0	209
30	23.8	19.7	1.21	73	4	192
35	21.9	9.7	2.26	147	53	75

[1] Ratio = $\frac{\text{Emergence of beets (coefficient of velocity)}}{\text{Growth rate of } R.\ solani \text{ (millimeters per day)}}$.
[2] Soil moderately infested.
[3] Soil heavily infested.

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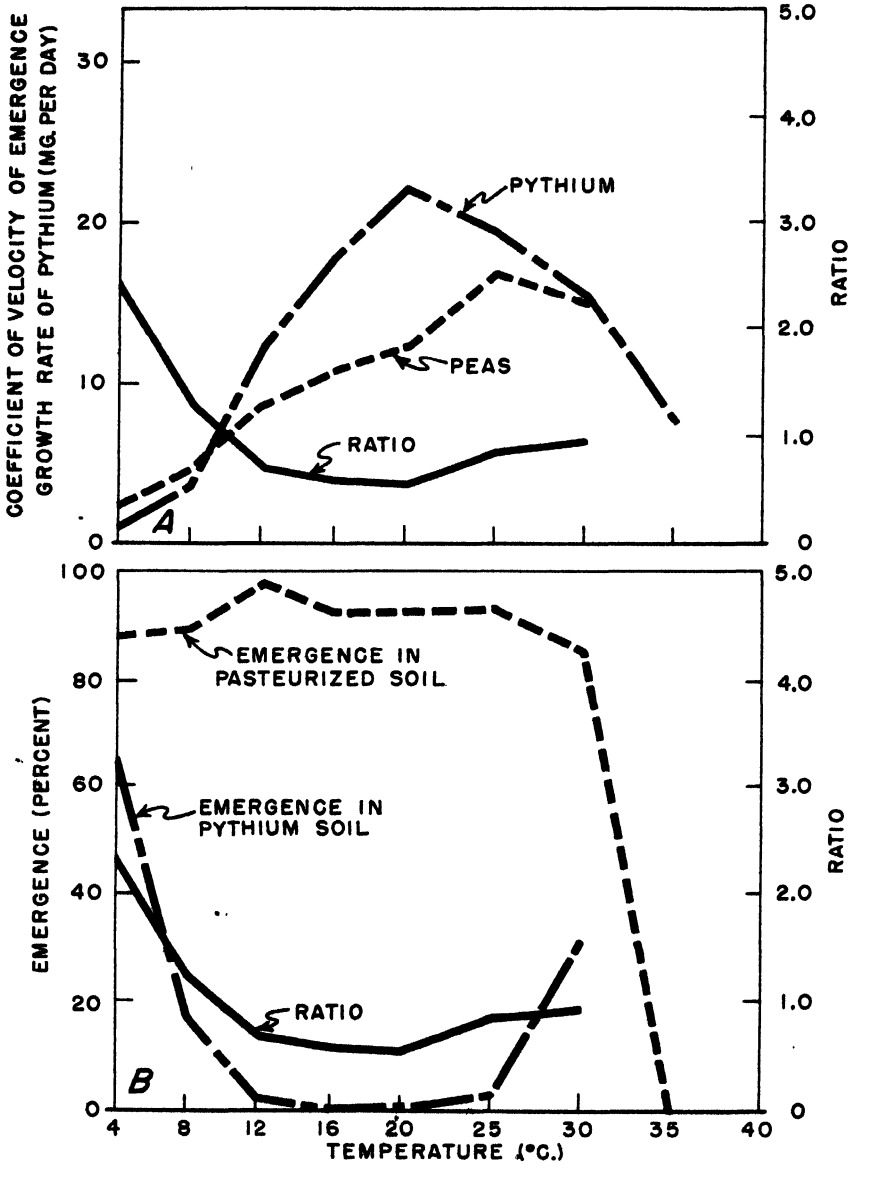

FIGURE 3.—*A*, Coefficient of velocity of emergence of garden peas, growth rate of *Pythium ultimum* in liquid medium, and ratio of emergence rate of peas to growth rate of *Pythium*; *B*, percent emergence of peas in pasteurized and in Pythium-infested soil compared with curve representing ratio of growth rates of host and pathogen reproduced from figure 3, *A*. Seed decay was less severe at high and low temperatures than between 12° and 25° C., a relation corresponding to the ratio between growth rates of host and pathogen.

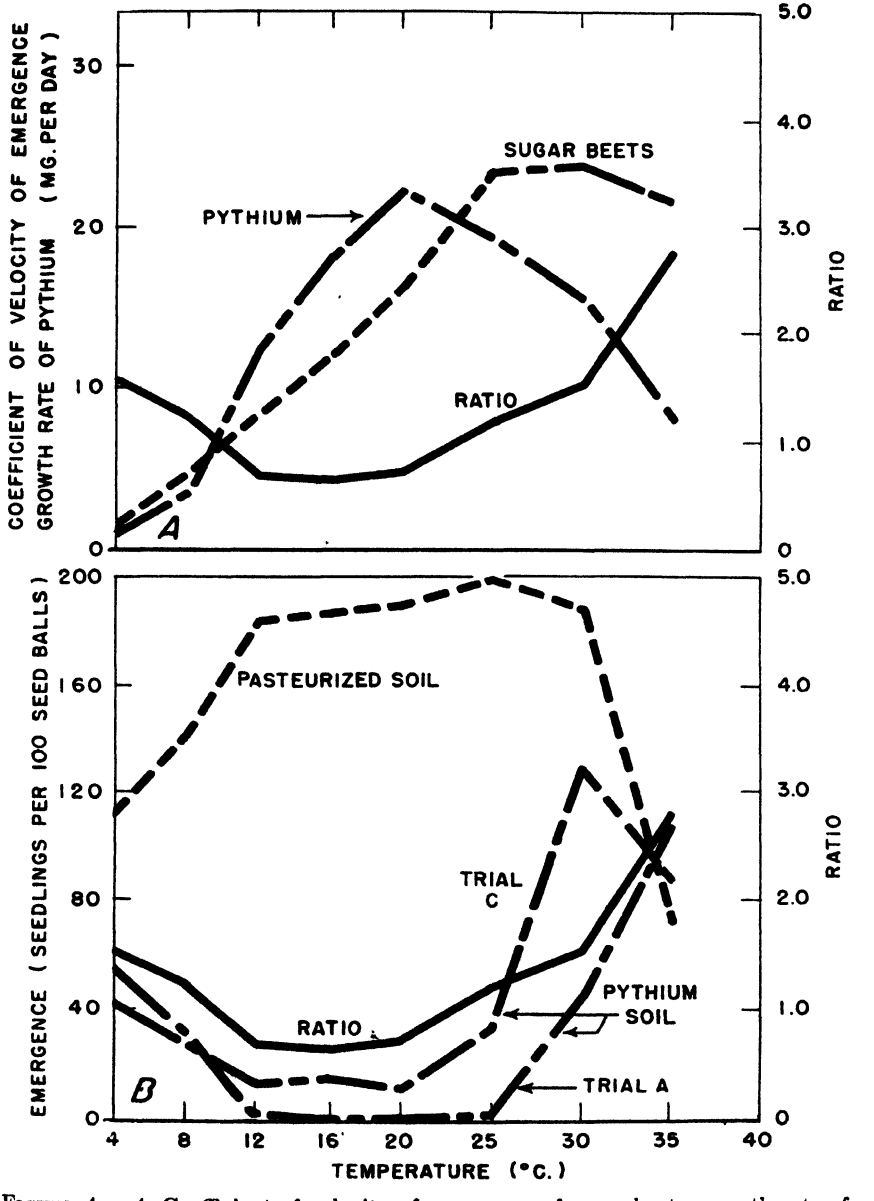

FIGURE 4.—*A*, Coefficient of velocity of emergence of sugar beets, growth rate of *Pythium ultimum* in liquid medium, and ratio of emergence rate of sugar beets to growth rate of *Pythium*; *B*, percent emergence of sugar beets in pasteurized soil and in soil infested by *P. ultimum* compared with curve representing ratio of growth rates of host and pathogen reproduced from figure 4, *A*. *Pythium* infection was severe throughout the temperature range favorable for germination of sugar beets.

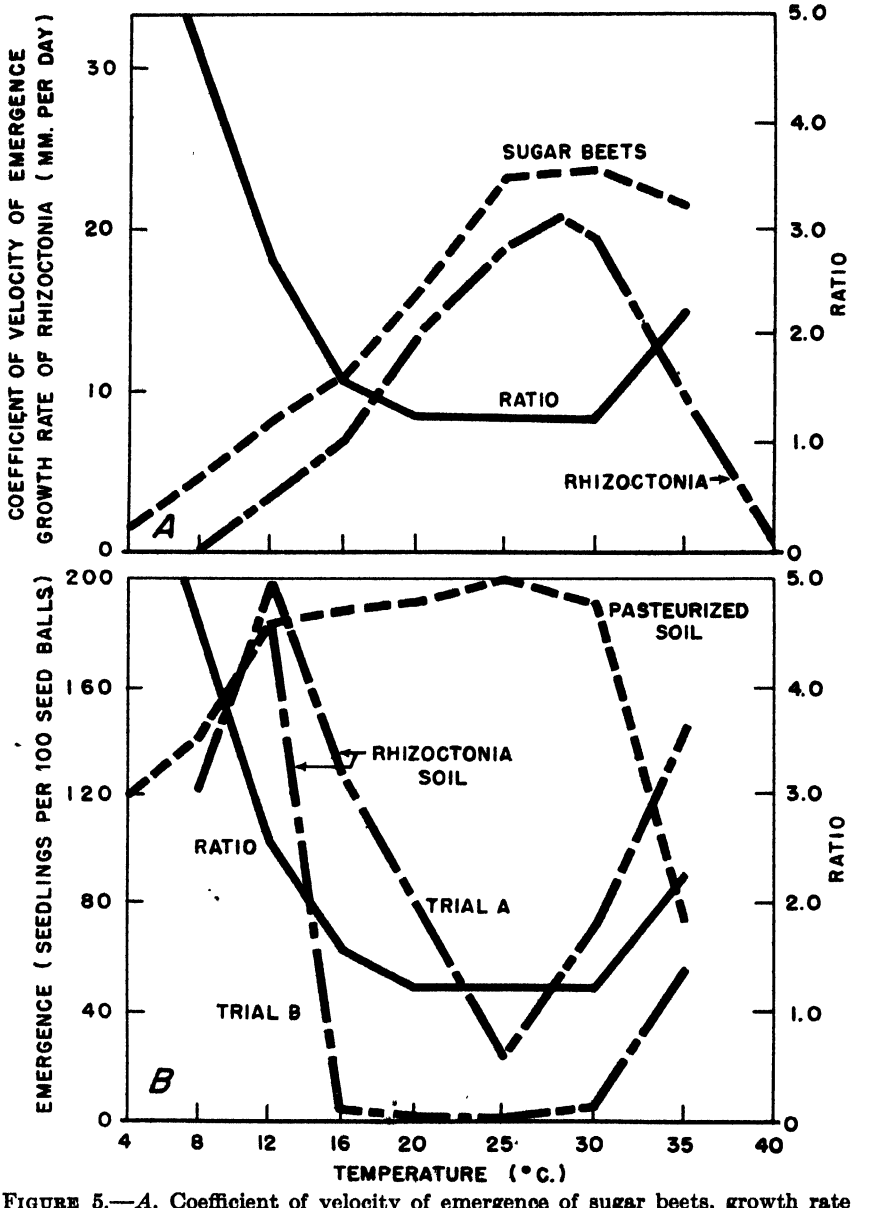

FIGURE 5.—*A*, Coefficient of velocity of emergence of sugar beets, growth rate of *Rhizoctonia* on agar, and ratio of emergence rate of sugar beets to growth rate of *Rhizoctonia*; *B*, percent emergence of sugar beets in pasteurized soil and in soil infested by *R. solani* compared with curve representing ratio of growth rates of host and pathogen reproduced from figure 5, *A*. Infection was severe between 16° and 30° C. with a maximum at 25°, near the optimum for both host and pathogen, but there was no infection at 12° or below where the host grew relatively faster than the fungus.

of the host to the growth rate of the pathogen (fig. 5, *B*). With a heavy soil infestation (trial B), infection was severe at 16° to 30°, but no preemergence damping-off occurred at 12° or below in either trial.

These results suggest that plantings at low temperatures would escape *Rhizoctonia* infection. Such a conclusion is supported by the fact that in central California this fungus is rarely recovered from early spring plantings but frequently from later ones.

PHOMA INFECTION OF SUGAR BEET

Phoma betae differs in one respect from the pathogens previously discussed. Instead of being soil-borne, it is carried either internally or externally on the beet seed balls produced in some areas. This fungus grows slowly at all temperatures, but a study of the relative growth of sugar beets and *Phoma* (table 8) shows that the higher the

TABLE 8.—*Relation of temperature to growth of Phoma betae and to emergence of Phoma-infected beet seed*

Temperature (°C.)	Emergence of beets, coefficient of velocity	Growth rate of *P. betae*,	Ratio of growth rates,[1] host to pathogen	Emergence in pasteurized soil of seedlings per 100 seed balls		
				Phoma-infected seed		Disease-free seed
				Lot A	Lot B	
		Milligrams per 24 hours		*Number*	*Number*	*Number*
4	1.6	0.7	2.28	3	--------	114
8	4.6	1 3	3.54	9	38	145
12	8.4	2 4	3.50	41	91	186
16	10.8	3 0	3.60	45	102	189
20	16.1	4 3	3 74	77	139	193
25	23.4	4.7	4 98	129	144	209
30	23.8	3.6	6.61	131	132	192
35	21.9	.4	54 75	68	--------	75

[1] Ratio=$\frac{\text{Emergence of beets (coefficient of velocity)}}{\text{Growth rate of } P.\ betae \text{ milligrams per day}}$.

temperature the more favorable it is for the host as compared with the pathogen.

With most seed lots *Phoma* produces chiefly postemergence root or hypocotyl infection and little or no preemergence damping-off. In a few heavily infected lots, however, preemergence infection was abundant. Two of these lots, A and B, were tested in pasteurized soil at 4° to 35° C. Each emerged as well at 25° or 30° as when the lots were disinfected before planting; but at low temperatures the emergence of *Phoma*-infected lots dropped much lower than that of disease-free seed lots at the same temperatures. As shown by table 8, the zone of severe preemergence infection corresponds to the lowest ratios between host growth rate and pathogen growth rate.

Low emergence of both infected and disease-free seed lots at 35° C. is characteristic of all plantings at that temperature.

PHYTHIUM INFECTION OF WATERMELON

In contrast to spinach and peas, watermelon is typical of high-temperature crops. Even in pasteurized soil (table 9) no seedlings emerged at 12° C. or below, only a few appeared at 16°, but excellent results were obtained at 20° to 35°. The highest emergence rate was found at 35°, with the rate decreasing as the temperature was lowered (fig. 6, *A*). The ratio of growth rates shows that at low temperatures *Pythium* grew relatively faster than watermelon, whereas at high temperatures this relation was reversed.

TABLE 9.—*Relation of growth rates of watermelon and Pythium ultimum to emergence in infested soil*

Temperature (° C.)	Emergence of watermelon, coefficient of velocity	Growth rate of *P. ultimum*,	Ratio of growth rates,[1] host to pathogen	Emergence in—			
				Pythium soil			Pasteurized soil
				Trial A [2]	Trial B [3]	Trial C [3]	
		Milligrams per day		*Percent*	*Percent*	*Percent*	*Percent*
4	0	1.0	0	---	---	---	---
8	0	3.6	0	---	---	---	---
12	0	12.2	0	0	0	0	0
16	3.6	17.2	.21	0	0	0	17
20	8.5	22.2	.38	20	0	1	94
25	21.1	19.4	1.09	64	1	6	90
30	28.7	15.6	1 84	73	19	8	92
35	33.0	8.0	4.12	98	91	[4] 75	96

[1] Ratio = $\frac{\text{Emergence of watermelon (coefficient of velocity)}}{\text{Growth rate of } P.\ ultimum \text{ (milligrams per day)}}$
[2] Moderately infested soil.
[3] Heavily infested soil.
[4] 33° C.

By comparing the emergence in pasteurized soil with that in moderately infested soil (trial A), one can see that preemergence infection was severe at 16° and 20° C., moderate at 25° and 30°, and totally absent at 35° (fig. 6, *B*). In heavily infested soil (trials B and C) severe preemergence infection occurred at temperatures as high as 30° but not at 35°. The unintentional lowering of the temperature from 35° to 33° resulted in some preemergence infection in trial C.

The severity of infection in each trial was closely related to the ratio of growth rates at that temperature (fig. 6, *B*).

These data strikingly resemble Arndt's results (*1*) with cotton seedlings in *Pythium*-infested soil. Comparison of Arndt's "time required for emergence" for cotton with the present data on the growth rate of *P. ultimum* (table 2) indicates that his ratios would be similar to those for watermelon and *Pythium* at the same temperature. Arndt's findings could therefore be explained on the same basis.

RHIZOCTONIA INFECTION OF WATERMELON

Although *Rhizoctonia solani* does not grow so well at low temperatures as *Pythium ultimum*, its growth rate exceeds that of watermelon seedlings below 25° C. Above that temperature, conditions are increasingly favorable to watermelon. A single trial in heavily

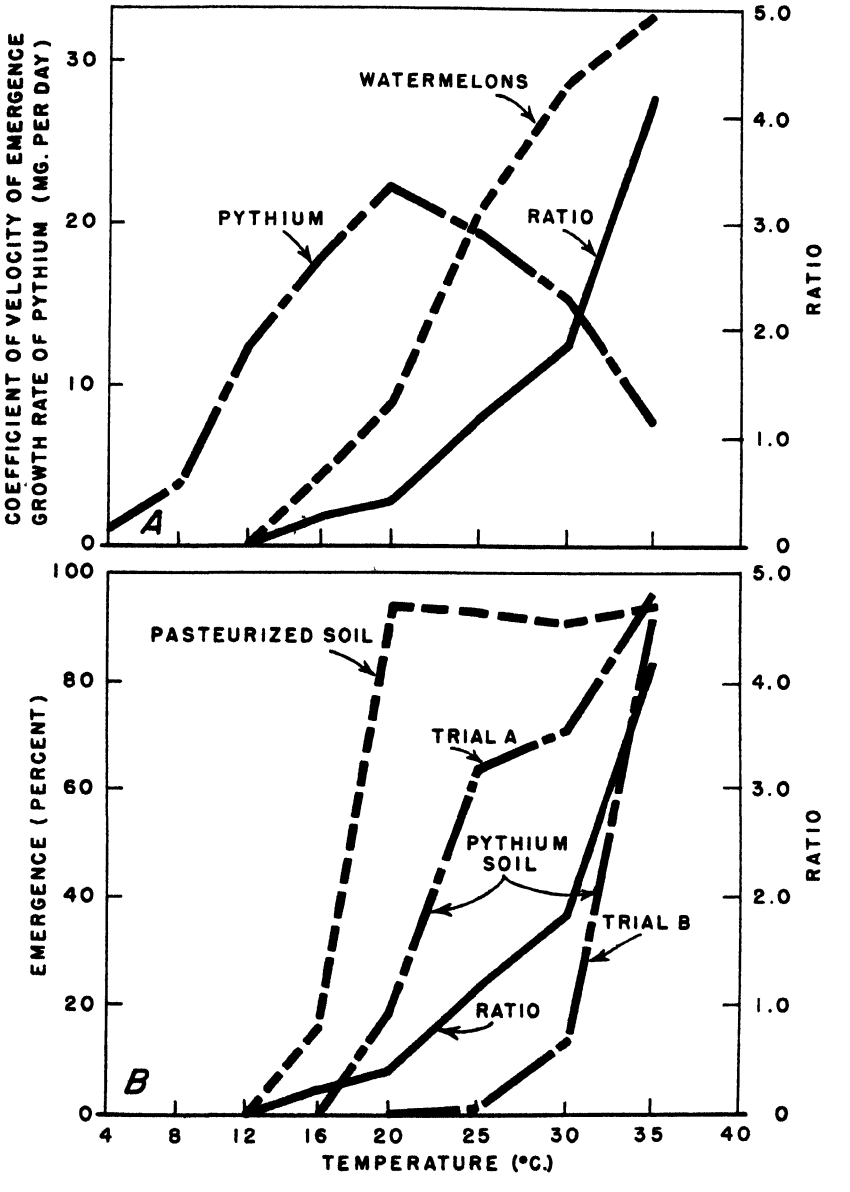

FIGURE 6.—*A*, Coefficient of velocity of emergence of a high temperature crop, watermelon; growth rate of a low temperature fungus, *Pythium ultimum;* and, ratio of the emergence rate of watermelon to growth rate of *Pythium*. *B*, Percent emergence of watermelon in pasteurized soil and in soil infested by *P. ultimum* compared with curve representing ratio of growth rates of host and pathogen reproduced from figure 6, *A*. Low temperatures favored the growth of the pathogen whereas high temperatures favored that of the host; damping-off decreased as the temperature rose.

infested soil (table 10) shows that *Rhizoctonia* causes severe seed decay and preemergence damping-off at 16° to 25°, but little if any preemergence infection at 30° and 35°.

TABLE 10.—*Relation of growth rates of watermelon and Rhizoctonia solani to emergence in infested soil*

Temperature (°C.)	Emergence of watermelon, coefficient of velocity	Growth rate of *R. solani*,	Ratio of growth rates [1] host to pathogen	Emergence in—	
				Rhizoctonia soil	Pasteurized soil
		Millimeters per day		*Percent*	*Percent*
4	0	0	--------	--------	--------
8	0	.1	0	--------	--------
12	0	3.1	0	0	0
16	3.6	6.9	.52	0	17
20	8.5	13.4	.63	0	94
25	21.1	19.0	1.11	0	90
30	28.7	19.6	1.46	83	92
35	33.0	9.7	3.40	90	96

[1] Ratio=$\frac{\text{Emergence of watermelon (coefficient of velocity)}}{\text{Growth rate of } R.\ solani \text{ (millimeters per day)}}$.

DISCUSSION

The comparisons in tables 3 to 10 and figures 1 to 6 illustrate eight combinations of hosts and pathogens. The severity of preemergence damping-off appears to be closely related sometimes to the growth rate of the host and sometimes to the growth rate of the pathogen. Neither of these factors alone adequately explains, as a rule, the relation between temperature and the severity of preemergence damping-off. However, in all combinations of host and pathogen tested, the ratio between the coefficient of velocity of seedling emergence and the growth rate of the pathogen is inversely related to the severity of infection. If this relation has general applications, then one can establish a temperature range within which infection is absent, a range within which infection is moderate, and one within which it is severe for any combination of host and pathogen, provided the growth rates of the two are known.

This concept explains why, in general, high-temperature crops like cotton, cowpeas, lima beans, and peanuts are more subject to seed decay or preemergence damping-off at low than at high temperatures, whereas low-temperature crops like spinach and peas often suffer less infection at low than at intermediate or high temperatures, provided soil moisture conditions are similar. By studying seasonal temperatures one is sometimes enabled to plant certain crops within escape periods for specific pathogens, or at least to avoid the period of most severe infection. If plantings of susceptible crops must be made during the period favorable for infection, then seed treatment is imperative; and one should select the specific treatment most effective against the organism or organisms most likely to operate at a given soil temperature.

In all combinations of host and pathogen, the lower the ratio of the growth rates, the more severe was the preemergence damping-off. For example, with sugar beets a rapid-growing fungus such as *Pythium*

ultimum shows a growth-rate ratio below 1.0 and causes severe preemergence infection at temperatures favorable to the fungus. With *Rhizoctonia solani*, a somewhat slower-growing fungus, the ratio of growth rates at temperatures most favorable to the fungus is between 1.0 and 2.0. At these temperatures *Rhizoctonia* produces less preemergence infection than *Pythium* but may cause considerable postemergence infection. With a slow-growing fungus, such as *Aphanomyces cochlioides*, sugar beets show a growth-rate ratio of 2.5 to 3.2 at the temperatures most favorable for the fungus. Infection by this organism is limited almost entirely to the postemergence phase, as reported by Buchholtz (*2*).

Apparently, therefore, ratios of below 1.0, indicating that the growth rate of the pathogen exceeds that of the host, are associated with the potentiality of severe preemergence infection. As the ratios increase from 1.0 to 4.0 the probability of preemergence infection is lessened, but postemergence infection may be severe. Ratios above 4.0 are associated with total absence of preemergence damping-off.

The present studies were limited chiefly to observations on preemergence infection, since the methods employed were not suitable for testing the effect of temperature on postemergence phases. Probably, however, the same relations hold for many types of infection on rapidly growing plant parts.

The occurrence of strains of a pathogen having different temperature requirements would alter these relations; and, when tested on the same hosts, a low-temperature strain of *Rhizoctonia solani* such as reported by Kadow and Anderson (*10*) would probaby not show the same temperature relations as the strain used in these trials.

The similarity between the ratio of growth rates and the severity of infection does not necessarily mean that growth rate is the controlling factor. In specific cases the susceptibility of seedlings has been shown to be correlated with chemical differences in the host brought about by temperature, as reported by Dickson and Holbert (*4*) and by Reddy (*18*). Investigations by McClure and Robbins (*15*) showed that resitance of cucumber seedlings to postemergence infection by *Pythium* was associated with cell-wall lignification, which in turn was influenced by age of seedlings, nitrogen nutrition, and light.

Probably, however, even in these cases, the physiological or anatomical changes in the host that limit infection are closely associated with the growth rate of the host and infection is related to the relative activity of the host and the pathogen.

SUMMARY

To determine the effect of temperature upon the severity of preemergence damping-off, spinach, sugar beets, peas, and watermelons were germinated in pasteurized soil and in soil infested by specific pathogens and maintained at controlled soil moistures and temperatures.

The coefficient of velocity of emergence was determined for each host from daily emergence counts in pasteurized soil at each temperature. The growth rate for each pathogen was determined at each temperature from measurements on agar plates or from nutrient solutions.

Spinach, a low temperature crop, was most severely infected in *Pythium*-infested soil between 12° and 20° C. and escaped preemerg-

ence infection only at 4°. In *Rhizoctonia*-infested soil, however, spinach suffered little or no preemergence infection at 12° or below, moderate infection at 16°, and severe preemergence damping-off at 20° or above.

Watermelon, a high temperature crop, escaped infection by either *Pythium* or *Rhizoctonia* at 35° C. but was more severely infected as the temperature was lowered.

In *Pythium*-infested soil, seed decay and preemergence infection of garden peas was most severe between 12° and 25° C.

The temperature ranges in which the most severe preemergence infection of sugar beets occurred were: *Pythium*-infested soil, 12° to 20° C.; *Rhizoctonia*, 16° to 30°; and from seed infected with *Phoma betae*, 4° to 20°.

In all combinations of host and pathogen preemergence infection was most severe at temperatures that were relatively less favorable to the host than to the pathogen as measured by the ratio of their growth rates.

From these trials it appears, therefore, that other factors being constant, the relative growth rates of the host and pathogen determine to a considerable degree the severity of preemergence infection at different temperatures.

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RESISTANCE OF LATHYRUS SPP. AND PISUM SPP. TO ASCOCHYTA PINODELLA AND MYCOSPHAERELLA PINODES[1]

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INTRODUCTION

The culture of the Austrian Winter field pea, a variety of *Pisum sativum* L., grown as a winter cover crop throughout the southeastern part of the United States and the Pacific Northwest, is considerably restricted by its susceptibility to diseases. Most destructive of these diseases in the Southeast are those caused by *Ascochyta pinodella* L. K. Jones, *Mycosphaerella pinodes* (Berk. and Blox.) Vest., and *Aphanomyces euteiches* Drechs. In the hope of discovering for use in breeding work a field pea more resistant than the Austrian Winter variety to these fungi a study was initiated in the autumn of 1935 at Experiment, Ga. During a 10-year period many different lots of *Pisum* and a few of *Lathyrus* were tested, many of them several times. Some of the difficulties involved and the methods of surmounting them were described in a preliminary report in 1940.[3] The present paper records the results of the studies of the species, varieties, and strains for resistance to *A. pinodella* and *M. pinodes* only. The investigations of *A. euteiches* are not entirely complete, and the results will be reported at some future date.

MATERIALS AND METHODS

The seeds used in these investigations were obtained in this country and from abroad. Few of the peas tested, other than the lots of the Austrian Winter variety, were able to survive the more severe of the winters at Experiment, Ga. It became necessary, therefore, to provide a measure of protection for the plants, especially during cold nights. Previously described hotbeds,[3] heated with electric cable and covered with heavy cloth, were found to be adequate. The seeds were planted during the first half of October in well-fertilized field soil in 6-inch pots. When available, 10 seeds were planted in each pot.

[1] Received for publication March 13, 1947. Cooperative investigations of the Division of Forage Crops and Diseases and the Georgia Agricultural Experiment Station. Paper No. 155, Journal Series, Georgia Agricultural Experiment Station.

[2] Grateful acknowledgment is made to Roland McKee, Division of Forage Crops and Diseases, to H. A. Schoth, Oregon State College, Corvallis, Oreg., and to the many others who supplied the seed used in these investigations.

[3] WEIMER, J. L. METHODS OF VALUE IN BREEDING AUSTRIAN WINTER FIELD PEAS FOR DISEASE RESISTANCE IN THE SOUTH. Phytopathology 30: 155–160, illus. 1940.

In order to insure abundant infection 2- to 4-inch plants were inoculated by placing on the surface of the soil 1 or 2 tablespoonfuls of autoclaved peas on which the fungus to be tested was growing. After the inoculum was applied, the pots were watered thoroughly and kept covered with the hotbed cloth for 2 days; this cover was wet down several times during that period.

Since 2 fungi (*Ascochyta pinodella* and *Mycosphaerella pinodes*) were involved, it was necessary to have 2 duplicate sets of pots, 1 set in each bed. Thus, in most experiments 40 plants were under test at the same time. When the results were not conclusive, the lots were included in the test the following year. Pots of the Austrian Winter variety, which was used as the control, were scattered throughout the beds.

Samples of seed were sometimes obtained after the general planting had been made. The plants from such seed often made poor growth until late spring. These small plants were almost always less severely diseased than those of the lots planted earlier.

In late spring, usually about the first of May, the best plants were taken from the hotbeds to a cloth enclosure,[5] and seeds were obtained from most of them. Seeds thus obtained were tested again in the hotbed the following season. The best plants were also used as parents in the breeding program.

When sufficient seeds were available, duplicate rod rows were planted in the field on land that had grown peas for two or more consecutive years. No attempt was made to inoculate the field-grown plants, and the amount of infection varied considerably from year to year, depending on the rainfall. In some years a large proportion of the plants growing in the field were killed by freezing and disease.

In addition to stand counts made in late autumn, notes were taken at irregular intervals, usually two or three times during the spring months. The final observations were made in late April or early May. The ratings made at this time were used as the best obtainable criterion of the resistance of the plants.

Hotbed space, seed, and labor were not available in sufficient quantity to permit the planting of enough replications to make a statistical study of the data worth while. The fact that the control pots in different parts of the same hotbed varied widely showed that a large number of replications would have been necessary to establish statistically significant differences. In view of these variations it was concluded that only the rating denoting the most severe infection could be accepted as indicative of the susceptibility of a variety. Often ratings were influenced by place effect, seasonal growth rate, and doubtless other factors not always recognized. When possible, doubtful ratings were checked in repeated hotbed tests as well as in field plots.

The plants were rated as follows: Immune, highly resistant, resistant, moderately resistant, susceptible, and very susceptible. No counts were made. The most important items considered in making the ratings were (1) killing of the plants, (2) extent of the damage to the lower part of the stem and percentage of stem involved, (3) num-

[5] See footnote 3, p. 181.

ber of basal leaves that had been killed, and (4) severity of spotting and amount of dead tissue in the upper leaves.

It was found that, even though the pots inoculated with the two fungi were kept in separate hotbeds, the distance between them was not sufficient to prevent the spread of the fungi from one bed to the other. Consequently there was a mixture of the two fungi in both beds, especially by late spring. Since the symptoms produced by the two fungi are so nearly alike, it was impractical to differentiate plants affected by them. Likewise, septoria leaf spot sometimes was prevalent early in the spring as a result of natural infection. It was impossible, therefore, to give to each lot of plants a rating that would denote its true reaction to either of the fungi tested. Such ratings, if desired, must be obtained by other experimental methods. The results presented in this paper must be considered as a composite reaction of the plants to inoculation with *Ascochyta pinodella* and *Mycosphaerella pinodes* and to a slight extent to natural infection with *Septoria pisi* West. Whenever possible, however, injury caused by *Septoria* was disregarded. From many viewpoints composite results of this nature are not the most desirable, but they represent exactly the condition found in the field. Since the objective of these tests was to find breeding stock that would survive this combination of diseases, the methods were considered suitable for the purpose.

SYMPTOMS USED IN RATING RESISTANCE

Although several thousand plants were inoculated and studied, there was not found any single symptom that would consistently differentiate the diseases caused by *Ascochyta pinodella* and *Mycosphaerella pinodes* under the conditions of these experiments. The only certain method of determining which fungus was present was to make isolations. The study of pycnidia often present on dead parts was also helpful. The symptom most commonly found early in the spring was a browning or blackening of the base of the stems, which usually started just below the surface of the soil. In very susceptible varieties the stem was decayed entirely through in a relatively short time and the plant died; in other varieties the damage was more superficial, and death came more slowly, if at all. Some plants tested were already killed, or nearly so, when the Austrian Winter plants showed almost no disease. There were all gradations between these extremes.

Another prominent symptom was leaf spot. Usually this did not develop in abundance until later in the spring, when it varied in severity, depending largely on weather conditions. The leaves were killed from the base of the plants upward. Sometimes 6 inches to 2 feet of the ends of the vines still had green leaves at the time the experiment was discontinued. In wet seasons all of the green leaves were more or less spotted, but when conditions were less favorable for infection some of the leaflets at the ends of the vines had few or no lesions.

The presence or absence of leaf spotting, especially late in the spring, was of doubtful value as an aid in rating the different lots. Many of the plants grew rapidly and produced long vines that climbed over the tops of the other plants or the sides of the bed where there were free air circulation and consequent rapid dissipation of rain and dew. Other plants grew slowly, and their tops always remained near the ground where the humidity was higher.

The spotting of pods was of no importance in this work, since the experiments were largely discontinued before pods were formed.

The plants of certain varieties grew rapidly throughout the season, whereas those of others grew slowly and made almost no growth during the colder winter months. In the former group were most of the English, or garden, peas (*Pisum sativum*); in the latter group were the more winter-hardy types such as the Austrian Winter peas. The diseases frequently appeared to make progress in proportion to the rate of growth of the plant, so that the stems of rapidly growing plants often quickly decayed and the plants were killed or nearly killed (fig. 1) at a time when the slower growing plants still remained

FIGURE 1.—Field pea plants from an inoculated imported lot of seed, typical of the rapid-growing type of plant, as they appeared on February 12, 1941. Practically all of the lower leaves had been killed, and large dark-brown lesions were present on the stems. All of the plants were dead on March 26. They were planted at the same time and treated the same as those shown in figures 2 and 3. × ½.

fairly free from disease (figs. 2 and 3). The slower growing lots in turn became severely diseased when they started rapid growth in the spring. The final result, insofar as the total amount of disease was concerned, was about the same by late spring, although some lots lived to produce much more green weight than others.

FIGURE 2.—Inoculated Austrian Winter field peas from a hotbed as they appeared on February 12, 1941. Some very small stem and leaf lesions caused by *Ascochyta pinodella* and *Mycosphaerella pinodes* were evident, and some of the lower leaflets had been killed by *Septoria pisi*. In general, however, the plants were still fairly healthy as compared with those shown in figure 1. × ½.

EXPERIMENTAL RESULTS

In some experiments a few isolated lots of *Pisum sativum* showed some resistance, but when retested they too were very susceptible to *Ascochyta pinodella* and *Mycosphaerella pinodes*. The named varieties and the unnamed strains of *Pisum sativum*, the unnamed strains of *Pisum* spp., and the lots of seed with numbers only that were rated as very susceptible follow.

Varieties of *Pisum sativum:*
Admiral (F. C.[1] 29931)
Advancer (F. C. 30049)
Agnes No. 7 (F. C. 29911)
Alah
Varieties of *Pisum sativum*—Con.
Alaska (F. C. 29932)
Alberta Blue (F. C. 29933)
Alcross (F. C. 30051)
Alderman (F. C. 30052)

[1] F. C. refers to accession numbers of Division of Forage Crops and Diseases.

FIGURE 3.—Inoculated plants of a double cross from the same hotbed as those in figures 1 and 2 as they appeared on February 12, 1941. These plants made slow growth and remained healthy much longer than the rapid-growing type shown in figure 1. They had been rated as very highly resistant to *Ascochyta* and *Mycosphaerella* on January 31; but, like the Austrian Winter plants shown in figure 2, they were rated as very susceptible on April 28. × ½.

Varieties of *Pisum sativum*—Con.
Alfred (F. C. 29934)
Allan Canner (F. C. 30053)
American Wonder (F. C. 30054)
Amraoti (F. C. 29935)
Andes (F. C. 29936)
Arabelle (F. C. 30073)
Archer (F. C. 29937)
Arthur (F. C. 29938)
Arthur 108 (F. C. 29939; F. C. 30055)
Austrian Winter[2]
Bangalia (F. C. 29940)
Belgium Sugar (P. I.[3] 137124)
Benah
Black Eye (F. C. 29913)
Black-eyed Marrowfat (F. C. 22431)
Bliss Everbearing
Blue Bantam (F. C. 30057)
Blue Imperial (F. C. 29942)
Blue Prussian (F. C. 29941; F. C. 30058)
Bothnia (P. I. 137174)
Brown Abyssinian
Canadian Beauty (F. C. 29943; F. C. 29944)
"Capucyners"

Varieties of *Pisum sativum*—Con.
Carlton (F. C. 29945)
Chancellor (F. C. 29946)
Chang (F. C. 29914; F. C. 30059)
Chinese Purple (P. I. 137118)
Clamart (F. C. 29915)
Colorado Marrowfat (F. C. 29947)
Concordia (P. I. 137175)
Cossacks (F. C. 29948)
Creole
Cudoiz
Daniel O'Rourke (F. C. 29949)
Dashaway (F. C. 30060)
Delano (F. C. 29950)
Desi (F. C. 29951)
Dwarf Telah
Earliest Perfection (F. C. 30063)
Early Britain (F. C. 29952)
Early Briton (F. C. 30061)
Early Perfectah
Early Perfection
Early Sabljas
Early Washington (F. C. 30062)
Early White (F. C. 29953)
Express
Farnham (F. C. 29956)
First and Best (F. C. 29957)
French Gray (F. C. 29916)

[2] 78 lots from different sources.
[3] P. I. refers to accession numbers of Division of Plant Exploration and Introduction.

Varieties of *Pisum sativum*—Con.
French June (F. C. 29958)
Friale (F. C. 29959)
Giant Edible Pod
Golden Marrow (F. C. 29917)
Golden Marrowfat (F. C. 30065)
Golden Vine (F. C. 22429)
Gradah
Gradus (F. C. 30066)
Green (F. C. 29961)
Green Scotch (F. C. 30067)
Greenville Nitrogen (F. C. 19003)
Gregory (F. C. 29962)
Grey Winter (F. C. 29963)
Gyllen (P. I. 137176)
Hanford's Canner (F. C. 30068)
Hangchow (F. C. 29964)
Harrison Glory (F. C. 30069)
Hawley's Improved (F. C. 30070)
Hero (P. I. 137177)
Home Delight
Horal (F. C. 30071)
Horsford Market Garden (F. C. 30072)
Hundredfold
Kaiser (F. C. 29970; F. C. 30074)
Killarney (F. C. 29969)
Kron (P. I. 137178)
Laxton Progress
Lima (F. C. 29918)
Lincoln (F. C. 31015)
Little Gem (F. C. 30075)
Little Marvel (F. C. 30076; F. C. 30077)
MacKay (F. C. 29971)
Maple Pea (F. C. 29922; F. C. 29920)
Marchioness (F. C. 29972)
Marcrosse (F. C. 29973)
Marrow
Marscot (F. C. 29974)
Marvel
Mash (F. C. 29975)
May
McAdoo
McKay Blackeye (F. C. 22430)
Merhaim
Meyer (F. C. 29976)
Minn. No. 95 (F. C. 29977)
Multiplier (F. C. 29978; F. C. 29979)
Nelson (F. C. 29980)
Ne Plus Ultra (F. C. 30080)
New Canadian Beauty (F. C. 29919)
New Perfection (F. C. 29981)
Nott's Excelsior (F. C. 30079)
Openshaw (F. C. 29982)
Ostgota Green (P. I. 137180)
Ostgota Yellow (P. I. 137181)
Ottawa (F. C. 30082)
Papago Indian Pea
Paragon (F. C. 29983)
Partridge (F. C. 29984)
Peluskchka (F. C. 29985)

Varieties of *Pisum sativum*—Con.
Pelusker (P. I. 137182)
Potter (F. C. 29986)
Premah
Premium Gem (F. C. 30086)
Prince of Wales (F. C. 30087)
Profusion (F. C. 30084)
Recordah
Red Pea
Rice Extra Early (F. C. 30090)
Rice No. 13 (F. C. 30088)
Rice No. 300 (F. C. 30089)
Rogers K
Sabljas
Sacton Progress (F. C. 30092)
Sand Pea (F. C. 93979)
Sato (F. C. 30091)
Scarlet
Scotch (F. C. 29987; F. C. 29988)
Scotch Blue (F. C. 29989)
Senator (F. C. 30093)
Shanghi (F. C. 29990)
Smiley (F. C. 29991)
Solo (P. I. 137183)
Stratagem (F. C. 30094)
Sugar Edible Podded
Sunrise (F. C. 30095)
Surprise (F. C. 30096)
Sweet Field Pea
Tall Grey Sugar (F. C. 30097)
Tall Telephone (F. C. 30098)
Telefon
Thracian Field Pea
Tom Thumb (F. C. 30099)
Torsdag II (P. I. 137184)
Victoria (F. C. 29993)
Vida (F. C. 29994)
V's No. 2 (F. C. 29995)
Walah
Warshauer (F. C. 29996)
Wellwood (F. C. 29997)
White Australian (F. C. 29998)
White Canada (F. C. 22424; F. C. 29999)
Willett's Wonder
Windsor (P. I. 137121)
Wisconsin Early Sweet (F. C. 30103)
Wisconsin Perfection (F. C. 30105)
Wisconsin Perien (F. C. 30104)
World's Pride (F. C. 30000)
Wyoming Wonder (F. C. 30106)
Yellow Admiral (F. C. 30001)

Unnamed strains of *P. sativum:*
A–15 (Iraq)
A–16 (China)
F. C. 30002
F. C. 30003
F. C. 30004
F. C. 30005
F. C. 30107
F. C. 30108
F. C. 30129
F. C. 30130

Unnamed strains of *P. sativum*—Con.
F. C. 30131
F. C. 30640
F. C. 30641
F. C. 30642
F. C. 30859
F. C. 31499
P. I. 19710
P. I. 24262
P. I. 92108
P. I. 125672
P. I. 125673
P. I. 125839
P. I. 125840
P. I. 131883
P. I. 131884
P. I. 134271
P. I. 134646
P. I. 135920
P. I. 137120
P. I. 137122
P. I. 137125
P. I. 138945
Wade's N802-2-1
Soil Conservation Service No. 20-489

Unnamed strains of *Pisum* spp.:
F. C. 30198
P. I. 126341
P. I. 141896

Lots of seed with numbers only:[4]
F. C. 60581
F. C. 60583
F. C. 60888
F. C. 60890
F. C. 60901
F. C. 60904
F. C. 60905
F. C. 60915
F. C. 60922
F. C. 60962
F. C. 61751
F. C. 62439
F. C. 87979
F. C. 87980
F. C. 87983
F. C. 90472
F. C. 90474
F. C. 90759
F. C. 91192
F. C. 93978

[4] 20 unnamed, unnumbered lots from different sources also tested.

Three lots of *Pisum elatius* Stev. (P. I. 120622, P. I. 120629, and P. I. 141891) were slightly resistant in the early stages of growth. The same was true of a wild species of *Pisum* from Turkey (probably also *P. elatius*, F. C. 30199). *Lathyrus tingitanus* L. was moderately resistant, *L. sativus* L. resistant, and *L. hirsutus* L. immune.

The fact that the strains of *Pisum elatius* were of the slow-growing type may partly explain their seeming resistance early in the season. Some plants of this species have been used in breeding experiments, and their progenies were tested over a period of several years. At present there is little evidence to support a conclusion that hybrids having *P. elatius* genes are any more resistant to *Ascochyta pinodella* and *Mycosphaerella pinodes* than others without them. In the present work it was necessary to obtain winter hardiness in a hybrid before it could be tested under field conditions. In order to breed winter hardiness into a line having *P. elatius* genes it was necessary to cross it and then backcross it with a winter-hardy variety. Even in such a cross a large proportion of the progeny was lost from freezing. In view of this complicating factor it may be desirable to explore the possibility of using this species as a source of germ plasm under other conditions.

There is real resistance in the genus *Lathyrus*, but all attempts to cross *Lathyrus* spp. with the Austrian Winter pea have failed.

DISCUSSION

It should be emphasized that the objective of the present study was to find a pea that is more resistant than the Austrian Winter to *Ascochyta pinodella* and *Mycosphaerella pinodes*. Whether it would have been practical, or for the present purpose desirable, to devise some method that might have made it possible to detect smaller differences in resistance, if such exist, may be debatable. Certainly considerable

difference in resistance was evident early in the course of the experiments; some plants were killed before others, such as the Austrian Winter and some of the new lines developed by breeding, had suffered any appreciable damage. These seemingly more resistant types, however, gradually succumbed as the season advanced, so that for the purpose of the present study there seemed to be little point in attempting to record these early differences.

It is true that the methods used put the plants to a very severe test, because the time over which the plants were exposed to the action of the fungi was very long, namely about 6 or 7 months as compared with about 2½ months required for many of the summer-grown garden and canning peas to complete their life cycle. The types of pea that remained dormant or grew more slowly during the winter months suffered little from disease during that period, whereas those that grew more or less all winter were correspondingly more severely injured by the fungi early in the season.

In general the writer's results agree with those of other workers. Seal [6] stated that he and Albrecht made a systematic search for species, subspecies, and varieties of *Pisum* and tested them for resistance to *Mycosphaerella pinodes*. So far as they could determine there was no marked difference in resistance to the fungi tested. After spending several years trying to develop a winter-hardy pea resistant to *Ascochyta pinodella* and *M. pinodes*, Ogden [7] concluded that he had found no strain resistant to these fungi. Noll [8] found that field peas (*P. arvense*) are more resistant to *A. pinodella* than are garden peas (*P. sativum*). Hare and Walker [9] tested 100 strains and varieties of *P. sativum* and concluded that no indication of practical tolerance to the ascochyta blight was found. Jones [10] tested the resistance of a large number of varieties of garden peas to *A. pinodella*, *A. pisi*, and *M. pinodes*. He found no varieties immune from any of these fungi but listed the Admiral 17.78, Advancer, Badger Special, Badger 20.140, Champion of England, Horsford, and Perfection as only slightly susceptible to *M. pinodes* and *A. pisi*. The Horsford Market Garden was the most resistant to all three of these fungi.

Ascochyta pisi, as well as *A. pinodella* and *Mycosphaerella pinodes*, produces ascochyta blight of *Pisum* spp. Since the Austrian Winter variety appears to be highly resistant to *A. pisi*, however, this fungus was not used in the present studies. Although it did produce a few lesions on the leaves of susceptible varieties as a result of natural infection, these lesions were never numerous enough to interfere with

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[6] SEAL, J. L. THE MYCOSPHAERELLA DISEASE OF WINTER PEAS, AND DISEASES OF WINTER PEAS AND VETCHES CAUSED BY ASCOCHYTA SPECIES. Ala. Agr. Expt. Sta. Ann. Rpt. (1937) 48: 24–25. [1938.]

[7] OGDEN, H. P. WINTER PEA BREEDING. Tenn. Agr. Expt. Sta. Ann. Rpt. (1935) 48: 11–12. [1936.]

[8] NOLL, W. ÜBER WEITERE BEFALLSYMPTOME UND MASSNAHMEN ZUR VERHÜTUNG VON SCHÄDEN DURCH ASCOCHYTA PINODELLA JONES, A. PISI LIB. UND MYCOSPHAERELLA PINODES (BERK. U. BLOX.) STONE BEI ERBSEN. Ztschr. f. Pflanzenkrank. 50: [49]–71, illus. 1940.

[9] HARE, W. W., and WALKER, J. C. ASCOCHYTA DISEASES OF CANNING PEA. Wis. Agr. Expt. Sta. Res. Bul. 150, 31 pp., illus. 1944.

[10] JONES, L. K. STUDIES OF THE NATURE AND CONTROL OF BLIGHT, LEAF AND POD SPOT, AND FOOTROT OF PEAS CAUSED BY SPECIES OF ASCOCHYTA. N. Y. State Agr. Expt. Sta. Bul. 547, 46 pp., illus. 1927.

note taking. Not all of the varieties listed as resistant to ascochyta blight by some writers were tested in the present investigations. It seems probable that such varieties as were tested failed to show resistance because of the severity of the method used and the long period over which the plants were exposed to the ravages of the disease.

SUMMARY

The results of 10 years of testing various varieties and strains of field and garden peas (*Pisum sativum*) have failed to disclose the existence of a pea that possesses any appreciably greater degree of resistance to *Ascochyta pinodella* or *Mycosphaerella pinodes* than does the Austrian Winter under the conditions of the experiments. Over 160 named varieties and many numbered and unnamed lots, some from several different sources, were studied. *Pisum elatius* and a wild pea from Turkey (probably *P. elatius* or a close relative) appeared to possess some resistance under the conditions of the experiments. Whether this was due to a transmissible character seems doubtful, since progeny from crosses in which these peas were used as a parent failed to show much if any increased resistance.

The strain of *Lathyrus hirsutus* used proved to be immune from the fungi studied, that of *L. sativus* was resistant, and that of *L. tingitanus* was moderately resistant. All attempts to cross these species of *Lathyrus* with the Austrian Winter pea failed.

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POLLEN DEGENERATION IN MALE-STERILE SUGAR BEETS, WITH SPECIAL REFERENCE TO THE TAPETAL PLASMODIUM[1]

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INTRODUCTION

When male-sterility in sugar beets (*Beta vulgaris* L.) (*4*)[2] is cytoplasmically inherited, completely male-sterile plants bear white, empty anthers. Normal pollen mother cells and normal microspores are produced, but the microspores fail to develop fully and disintegrate by the time the flowers open.

In the semi-male-sterile types, small, nonviable pollen grains are formed but the anthers usually do not dehisce. Sometimes viable pollen is produced by some branches of the inflorescence, and occasionally white anthers and yellow ones are borne within the same flower (*4*).

Pollen abortion, especially in hybrids of plants with different chromosome numbers, usually is the result of abnormal meiotic divisions; in sugar beets, however, the writer observed a different type of pollen degeneration in which the anther tapetum, through the development of a plasmodium, plays an important role.

Tapetal plasmodia and less prominent forms of the same phenomenon are not uncommon and may be regarded as normal in those plants in which they have been described (*6*), but as a pathological condition they seem to have been mentioned but once in literature (*7*). In sugar beets the tapetal plasmodium dominates the pathological picture from its inception to the destruction of the microspores.

MATERIALS AND METHODS

Terminal branches of sugar-beet inflorescences, fixed in Carnoy's fluid, were sent to the writer by F. V. Owen, of the United States Department of Agriculture Sugar Plant Laboratory, Salt Lake City, Utah. The material was taken from beets exhibiting cytoplasmically inherited male-sterility. For comparison, male-sterility not cytoplasmically inherited was also studied. The completely male-sterile and semi-male-sterile forms were derived by Owen "directly or indirectly from the sugar-beet variety U. S. 1 . . . , which was the first of the curly-top-resistant sugar-beet varieties released by the United States Department of Agriculture" (*4, p. 423*).

[1] Received for publication February 13, 1947.

[2] Italic numbers in parentheses refer to Literature Cited, p. 197.

The usual methods of dehydrating and embedding in paraffin were employed. The sections were cut 5μ to 10μ thick and stained with Heidenhain's iron-alum haematoxylin and chromotrope.

All photomicrographs were taken on Wratten M plates with B 58 and E 22 filters used singly and in combination.

THE ANTHER TAPETUM DURING NORMAL MICROSPOROGENESIS

Since microsporogenesis in normal sugar-beet flowers has been described in detail elsewhere (*1*), the discussion here will be limited to the behavior of the tapetum, which plays a major role in pollen abortion of male-sterile sugar beets.

A cross section through a young anther lobe (pl. 1, *A*) shows a central strand of dark-staining primary sporogenous tissue surrounded by a series of parietal wall layers. The first periclinal division in the primary parietal cells gives rise to two layers (pl. 1, *A*), which divide once more in a periclinal fashion. The parietal tissue is composed of a tapetal layer, two or more middle layers, and the endothecium, lying just beneath the epidermis. The cells of the endothecium enlarge late in ontogeny and develop the characteristic fibrous thickenings (pl. 1, *F*), which are related to the dehiscence of the anther.

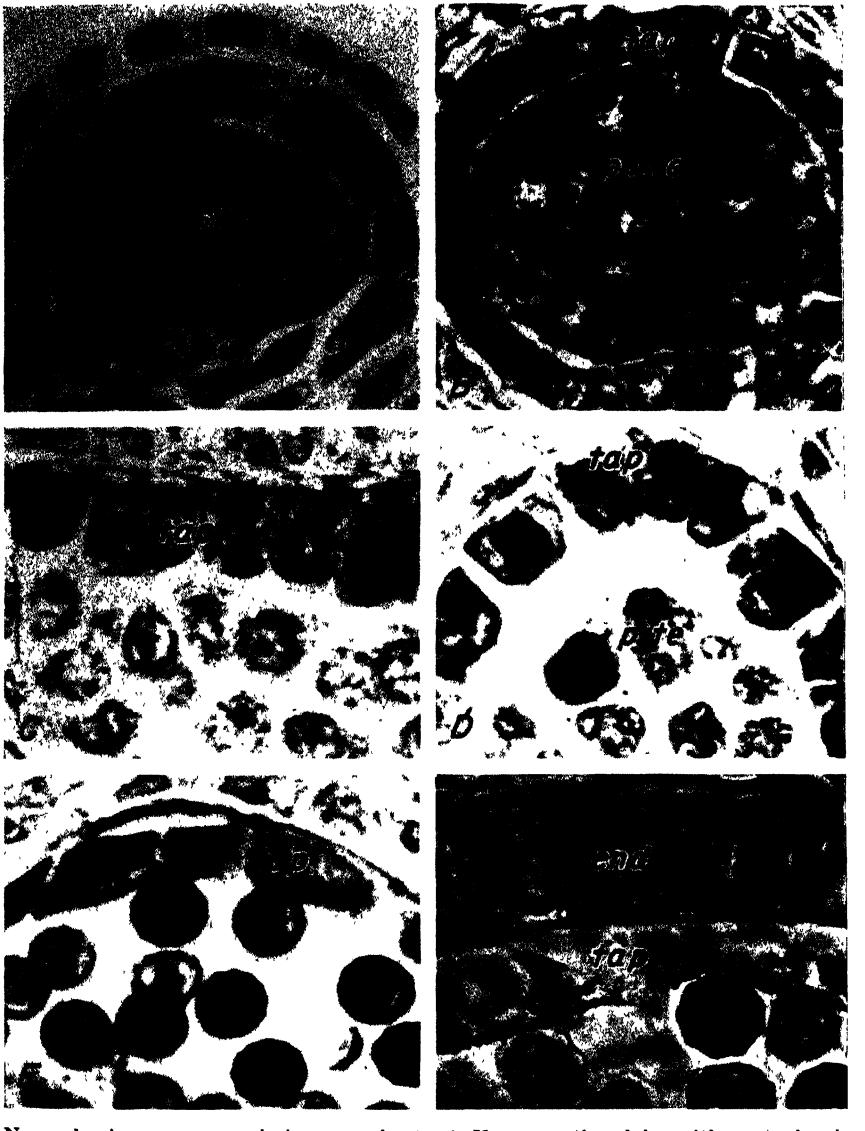
Although there is much controversy in literature concerning the origin of the tapetum, in sugar beets it appears to be derived from the inner parietal wall layer since the primary sporogenous tissue (pl. 1, *A*) is already well differentiated before the second periclinal division in the cells of the parietal tissue takes place. The cells of the young tapetum are nearly isodiametric (pl. 1, *B*). The single nucleus is large and almost fills the cell lumen. Beginning with synizesis, the tapetal cells enlarge radially and become binucleate (pl. 1, *C*). Each nucleus contains one large nucleolus and parietal or diffuse chromatin granules. Some of the tapetal cells may contain compound nuclei (pl. 1, *C*, *D*). The cytoplasm is very dense and stains more intensely than that of the pollen mother cells.

The nuclei and cytoplasm of the tapetum indicate strongly a metabolic relation between tapetum and developing microspores. This active phase is maintained throughout the two meiotic divisions and the liberation of the microspores from the tetrads. With the progressive enlargement of the anthers and the growth of the pollen grains, the tapetal cells become tangentially elongated, the nuclei undergo chromatolysis, and the nuclear substance mingles with the degenerating cytoplasm (pl. 1, *E*). In anthers ready to dehisce the tapetum has completely disappeared or may be seen as a pale narrow band lining the anther cavity (pl. 1, *F*). Remains of small, shrunken nuclei may also be discerned.

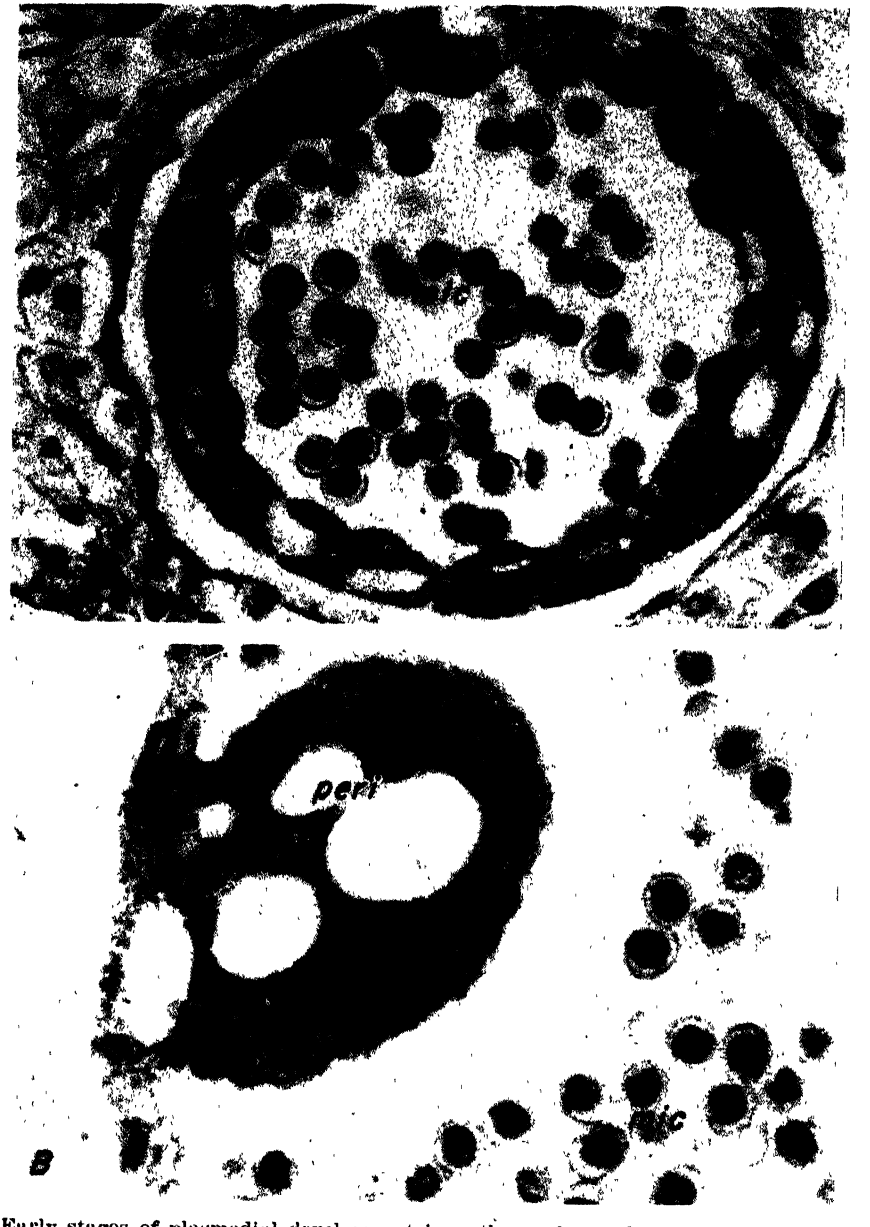
POLLEN ABORTION IN COMPLETELY MALE-STERILE SUGAR BEETS

CYTOPLASMICALLY INHERITED TYPE ASSOCIATED WITH A TAPETAL PLASMODIUM

In many of the lower plants and a number of angiosperms the walls of the tapetal cells break down and allow the protoplasts to coalesce and form a tapetal plasmodium (*5*). This flows in among the immature spores and contributes to the development of their coats.



Normal microsporogenesis in sugar beet: *A*, Young anther lobe with central primary sporogenous tissue and wall layers; *B*, older anther showing differentiation of tapetum; *C*, binucleate tapetum during synizesis of pollen mother cells; *D*, binucleate tapetum during formation of tetrads; *E*, degenerating tapetum and pollen grains with thickened exine; *F*, completely degenerated tapetum, endothecium with wall thickenings, and mature pollen. × 850. *an w*, Anther wall; *end* endothecium; *p m c*, pollen mother cell; *p tc*, pollen tetrads; *sp t*, sporogenous tissue; *tap*, tapetum.



Early stages of plasmodial development in anthers of cytoplasmically inherited male-sterile sugar beets: *A*, Early stage in the development of periplasmodium; *B*, somewhat later stage with periplasmodium making pseudopodiumlike incursions into anther cavity; note large nuclei and vacuoles. × 850. *mic*, Microspores; *peri*, periplasmodium.

In the monocots investigated by Clausen (*2*) there are distinct types of plasmodial formations sufficiently different to have taxonomic importance. In many forms the tapetal cells, after the walls separating them have been dissolved, make pseudopodiumlike incursions into the anther cavity; a common plasmodium that surrounds the pollen grains is finally formed. Embedded in the plasmodium are the nuclei of the tapetum. When the pollen grains have developed the exine, the plasma of the plasmodium diminishes and the nuclei begin to degenerate; the chromatin threads break up and become pale; and finally only pollen grains remain.

A case of plasmodium formation in connection with pollen degeneration in *Ranunculus acris* L. has been described by Whyte (*7*, *p. 187*). In the hermaphrodite normal plant there is a considerable interval in the reduction processes in anthers and ovules in a given flower. Where the reduction divisions occur almost simultaneously, the tapetum in the anthers fails to function and cessation of pollen formation results. As a direct consequence, the cells of the tapetum " . . . hitherto normal . . . break apart leaving their usual situation along the wall of the pollen sac; coalescence of several cells may then take place forming large plasmodial masses . . . the whole occupying in many instances the greater part of the pollen sac." Here then is an instance where a normally useful plasmodium causes irreparable injury to the pollen.

The cytology of meiosis in male-sterile sugar beets agrees in every detail with that in normal sugar beets. The young microspores may still be associated in the tetrads when the cells of the tapetum begin to behave in an abnormal manner. The boundaries of the tapetal cells break down, and the contents flow together to form a periplasmodium (pl. 2, *A*).

From its early development to its degeneration the cytoplasm of the plasmodium is very dense and contains conspicuous vacuoles. The nuclei are large (pl. 2, *B*) and often are bunched in great numbers (pl. 3, *A*), suggesting an increase over the number present in the original cellular tapetum. The nuclei are round, narrow oval, or sometimes lobed. Most of them contain one large nucleolus and peripheral chromatin granules of various sizes. Some nuclei appear definitely compound, containing two or three large nucleoli.

Most of the young microspores appear normal. Each microspore has a large nucleus embedded in dense cytoplasm which, perhaps as a result of fixation, is slightly shrunken away from the spore wall (pl. 2, *B*). There is a single large nucleolus. In some of the dark-staining microspores there may also be seen large chromatin granules resembling typical chromocenters. Some of the microspores have become pycnotic and stain black. In a few of these a dark-staining nucleus containing large chromatin granules is discernible under intense illumination. The spore wall is very thin, and it fails to thicken even late in ontogeny. Some microspores are represented by spore membranes only, all cell contents having disappeared.

The plasmodial jacket increases in width unequally. Here and there it bulges prominently into the anther cavity (pl. 2, *B*). These broad pseudopodiumlike incursions never become detached from the main jacket and never surround the microspores as they do in plants in which the appearance of a plasmodium is considered a part of normal microgenesis.

With the growth of the plasmodium the microspores are crowded into the narrowing lumen of the anther (pl. 3, *A*). So far there has been no noticeable structural change within the microspores except that additional ones have become pycnotic. There is no increase in thickness of the spore membrane.

After the plasmodium has attained maximum development, it begins to degenerate. The cytoplasm is first affected. It becomes coarsely alveolar (pl. 3, *B*) and then stringy (pl. 4, *B*). In its alveolar state it stains rather light; but later it retains the stain more tenaciously (pl. 4, *A*), and the cytoplasmic strands finally stain black (pl. 4, *B*). The large nuclei of the plasmodium are at first little affected (pl. 3, *B*), but with increased destruction of the cytoplasm they also exhibit signs of degeneration. At the beginning there is an increase in the number of peripheral chromatin granules in the nucleus and a deeper staining reaction (pl. 4, *A*), but they retain a certain degree of organization even after the complete disorganization of the cytoplasm.

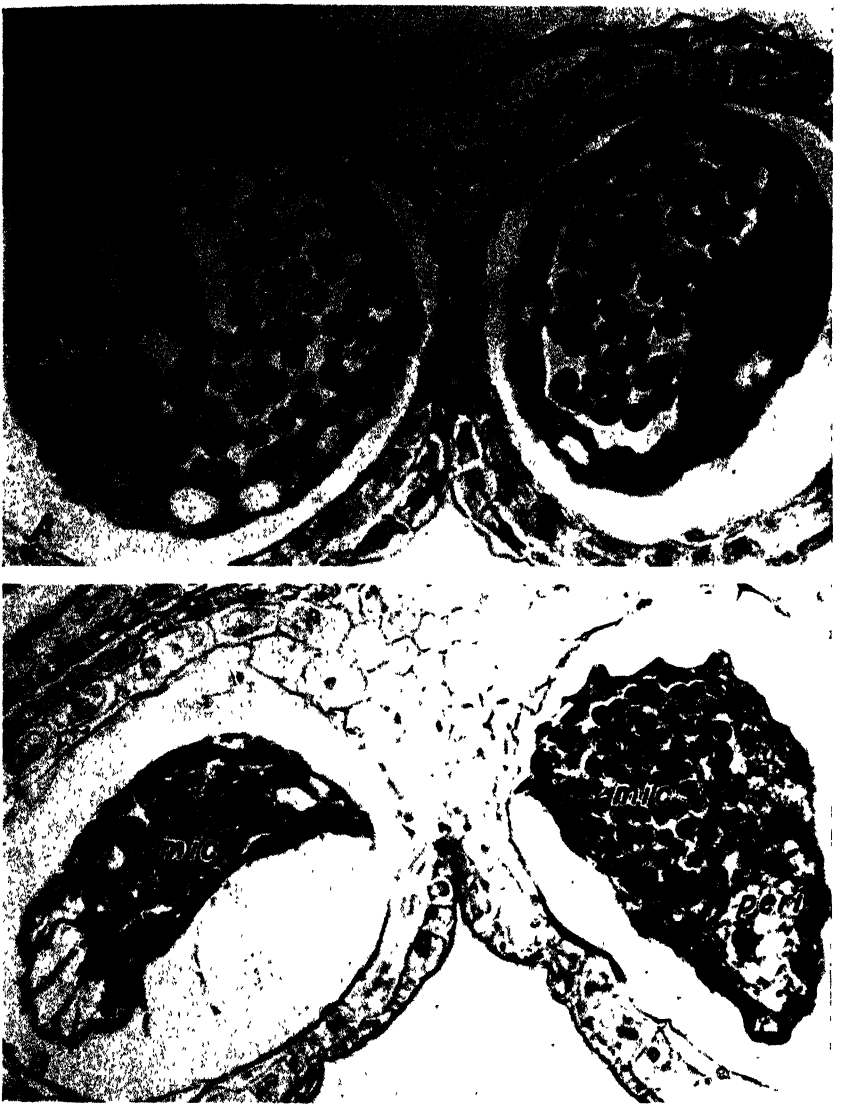
According to Kostoff (*3*), evidently only the cytoplasm possesses the ability of self-regulation, being able to extract foreign substances and those not of immediate use and to deposit them in vacuoles. The nucleus is always more susceptible to foreign agents, and it is killed by poison before the cytoplasm is irreparably injured. When the plasmodium in male-sterile sugar beets is in its ascendancy, the large vacuoles, which are a notable feature of the organization of the plasmodium (pl. 2, *B*), may be places of storage for excess waste products resulting from unusual metabolic activities. These waste products may be released with the advent of plasmodial degeneration and may hasten the destructive processes which begin after the peak of plasmodial development has been reached. According to Kostoff, one would expect an early destruction of the nuclei, but the cytoplasm deteriorates before there are recognizable pathological changes in most of the nuclei. Nevertheless it seems that with the disappearance of the protecting vacuoles the cytoplasm would become more susceptible than the nuclei.

The microspores continue to show little damage even though they become increasingly compressed and in their entirety take on a honeycomb structure (pl. 5). With the complete disintegration of the plasmodium the microspores are finally destroyed. The anther cavity becomes empty except for a strand of blackened matter, which occasionally still shows evidence of cellular structure.

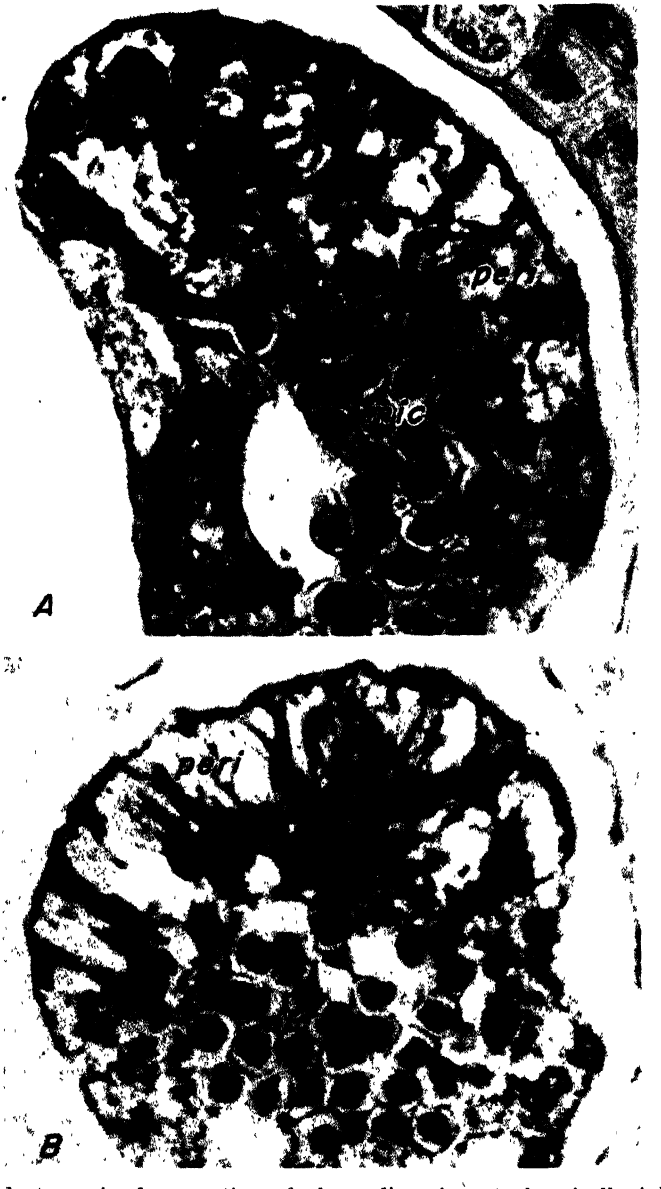
CYTOPLASMICALLY INHERITED TYPE ASSOCIATED WITH A CELLULAR TAPETUM

Pollen abortion not associated with a tapetal plasmodium frequently occurs; but while the flowers of a cluster may exhibit both types of degeneration, within a given flower only one type prevails.

In cases not involving a plasmodium, the tapetum remains cellular. It forms either a narrow lining (pl. 5, *A*, *B*) in which the individual cells may be stretched tangentially (pl. 5, *B*) or a very broad jacket with the cells meeting almost in the center. The former type is more common. Under low magnification the anther cavity appears large, round, and partly filled with microspores in various stages of degeneration (pl. 5, *C*). The tapetal cells are completely filled with fine gran-



Comparative stages of plasmodial development in anthers of cytoplasmically inherited male-sterile sugar beets. *A*, Periplasmodium at peak of development; note that some of the microspores are normal, while others are degenerate. × 450. *B*, Degeneration of plasmodium more advanced in the left anther lobe than in the right one. × 385. *mic*, Microspores; *peri*, periplasmodium.



Advanced stages in degeneration of plasmodium in cytoplasmically inherited male-sterile sugar beets. *A*, Degeneration in anther plasmodium; note that some of the plasmodial nuclei stain black and that the microspores in the center are compressed in such a way as to resemble a honeycomb cellular structure. *B*, Final stage in degeneration of plasmodium; the cytoplasm has become stringy, the nuclei are disorganized and black, and the microspores in the center are compressed. × 850. *mic*, Microspores; *peri*, periplasmodium.

Types of cellular tapetum and pollen degeneration in anthers of cytoplasmically inherited male-sterile sugar beets. *A*, Microspores already degenerated in tetrads. *B*, Most of the microspores degenerated and the tapetal nuclei flattened tangentially. × 850. *C*, Only relatively few microspores present, more than half having degenerated while still in tetrads. × 700. *tap*, Tapetum.

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Pollen degeneration in noncytoplasmically inherited male-sterile sugar beets: *A*, Anther with cellular tapetum and fully developed endothecium; *B*, Anther with tapetum and pollen completely degenerated. × 850. *end*, Endothecium; *mic*, microspores; *tap*, tapetum.

ular cytoplasm, in which the nuclei are barely discernible. Vacuoles, which are a prominent feature of the plasmodium, are wanting. The nuclei are small or medium large and usually compressed tangentially (pl. 5, *B*). The tapetal cells at the ends of the anther are shorter, and the nuclei are larger and almost spherical. Most nuclei have a very large nucleolus and a varying number of chromatin granules; some have several nucleoli and are definitely compound (pl. 5, *A*).

Some of the young microspores appear normal and look like those developing in association with the periplasmodium, but the majority have become pycnotic and stain black (pl. 5, *B*, *C*).

Degeneration of the microspores begins rather early, sometimes before they are liberated from the tetrads (pl. 5, *A*). The affected microspores, including those still in tetrads, are completely black, but under intense illumination a nucleus is still discernible in some of them. The outer margin of the blackened microspore is finely serrate or prickly and slightly retracted from the thin, hyaline spore wall.

Progressive degeneration of nuclei and cytoplasm in the tapetal cells is like that observed in normal microgenesis, so that it is difficult to ascribe to the tapetum any harmful influence on the developing microspores. But whatever the cause is, the effect is swift and lethal and there are seldom intermediate stages. Death often overtakes the spores while they are still in the tetrads, and those that are affected later show little or no growth. The cells of the endothecium remain small and fail to develop the characteristic wall thickenings.

NONCYTOPLASMICALLY INHERITED TYPE OF MALE-STERILITY

Anthers from male-sterile plants of the noncytoplasmically inherited type develop the endothecium normally (pl. 6), probably because degeneration of the microspores is initiated later in ontogeny and proceeds more slowly so that the anther wall has had no time to mature. The tapetum of such anthers always remains cellular, but the cells composing it enlarge greatly (pl. 6, *A*). The microspores enlarge slightly and even develop a thin exine. Cytoplasm and nuclei stain weakly and show signs of degeneration. In some microspores the contents are blackened and contracted into a spherical mass. At a later stage the anthers collapse; the anther cavity appears empty except for a dark strand of disintegrating microspores (pl. 6, *B*).

POLLEN DEGENERATION IN SEMI-MALE-STERILE SUGAR BEETS

According to Owen (*4*), the semi-male-sterile types apparently carry the same type of cytoplasm as that carried by completely male-sterile sugar beets, but their appearance is modified by one or more Mendelian factors. Environmental conditions have a marked influence on the expression of the semi-male-sterile condition, and classification for degree of semi-male-sterility may be subject to considerable variation.

A characteristic feature of pollen sterility in semi-male-sterile sugar beets is the lack of uniformity in the pathological picture even within a single flower (pl. 7, *A*). Some anthers are affected so completely that even the anther wall is destroyed, whereas others of the same flower are almost normal. In some anthers the tapetal cells have enlarged radially so greatly as to occlude the anther cavity. These cells

(pl. 7, *B*) have in the cytoplasm large, round nuclei and vacuoles like those of the periplasmodium. This type of tapetum is sometimes unilateral, prominent on one side and completely lacking or underdeveloped on the other. The microspores within such anthers show various stages of degeneration from a nearly normal condition to advanced pycnosis.

The anthers of many flowers appear normal except that the pollen grains, though uniform, are relatively small. Pollen grains in others approach normal size, but many of them are diseased (pl. 7, *C*).

Plasmodia apparently are not formed in anthers of semi-male-sterile sugar beets, but the large nuclei and cytoplasmic vacuoles in the radially elongated tapetal cells closely resemble those of the plasmodium.

DISCUSSION

What are the causative factors in the development of the periplasmodium and why is it restricted to sugar beets that exhibit cytoplasmically inherited male-sterility? The cytology of the young plasmodium suggests hypermetabolic activity, but not to the benefit of the developing microspores. The plasmodium seems to develop with structural continuity even though it makes pseudopodiumlike incursions into the anther cavity. What causes the precipitous catabolism after a developmental peak is attained is a moot question. It may be exhaustion of food and nutrient reserves or a sudden release of metabolic waste products presumably stored in the large vacuoles. Degeneration processes are more noticeable here than in normal plasmodia that help nourish the microspores and are themselves finally absorbed.

Still more puzzling is the question of selectivity which incites or inhibits the development of the plasmodium in different flowers of the same plant or the same flower cluster. Quite likely small inner environmental influences suffice to establish the choice, and once the impetus is given it carries on under its own momentum.

The anther plasmodium may prove to be a valuable tool in the elucidation of cytoplasmic inheritance in its pure form or in association with Mendelian factors, especially if used on genetic material grown in a controlled environment.

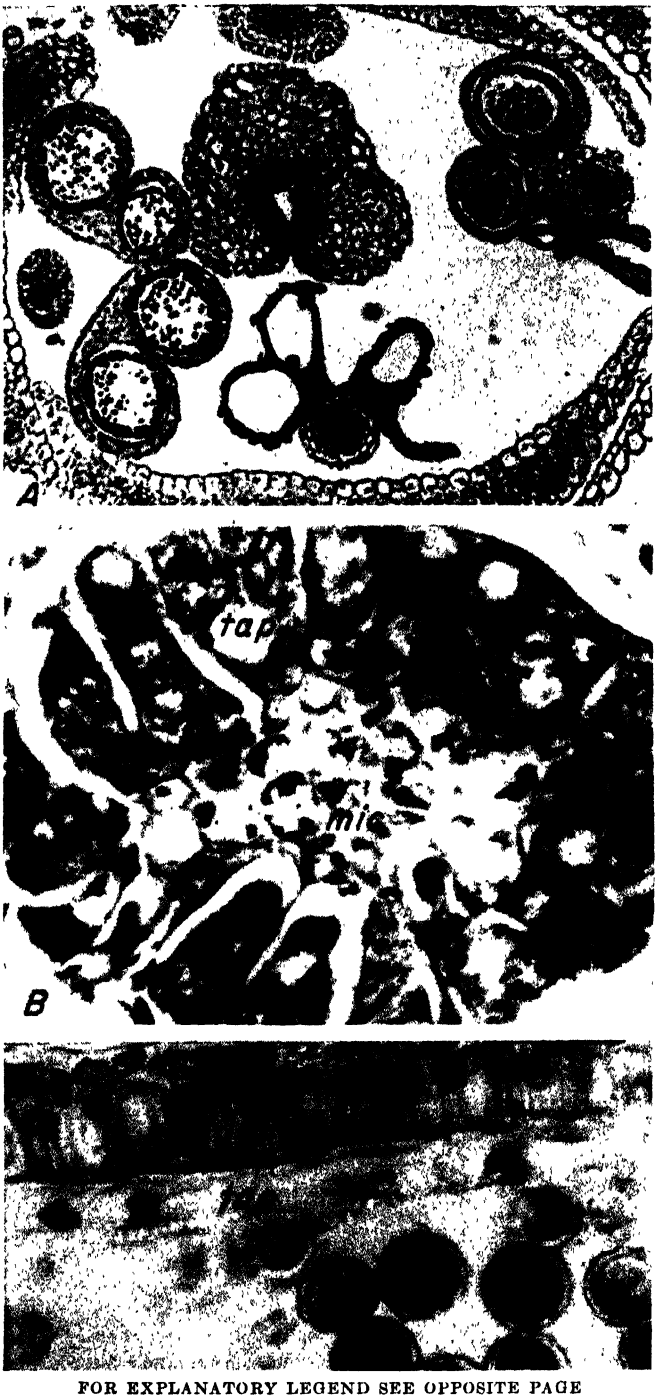
SUMMARY

Pollen abortion in anthers of sugar beets with cytoplasmically inherited male-sterility is associated with either a periplasmodium or a cellular tapetum. Both types may occur within a flower cluster but not within a single flower. The presence of a plasmodium somewhat delays pollen abortion, but where the tapetum remains cellular some microspores are destroyed while still in tetrads and there are seldom intermediate stages of significant duration.

The anatomical picture of pollen degeneration in semi-male-sterile

EXPLANATORY LEGEND FOR PLATE 7

Pollen degeneration in cytoplasmically inherited semi-male-sterile sugar beets. *A*, Cross section of flower; note that both contents and anther wall of one anther are completely destroyed. × 90. *B*, Cross section of an anther showing the circle of hypertrophied tapetal cells enclosing a few degenerated microspores in center. × 850. *C*, Diseased, black-staining pollen grains and normal ones side by side in the same anther. × 850. ***end***, Endothecium; ***mic***, microspores; ***tap***, tapetum.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE

sugar beets shows much variation and departs considerably from that exhibited by the completely male-sterile forms. The effect is at once severe and localized, involving at times both microspores and anther wall. A plasmodium never develops, although the cells of the tapetum become hypertrophied and may fill the anther cavity. Such hypertrophied cells have large nuclei and vacuoles like those in the periplasmodium.

In anthers from male-sterile sugar beets of the noncytoplasmically inherited type degeneration of the microspores seems to be delayed. Here the anthers, while showing only blackened contents, have a mature endothecium with fibrous wall thickenings well developed. The tapetum remains cellular. The cells composing it enlarge greatly, but they disintegrate at the time the microspores are destroyed.

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GROWTH RESPONSES OF TOBACCO SEEDLINGS IN ASEPTIC CULTURE TO DIFFUSATES OF SOME COMMON SOIL BACTERIA[1]

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INTRODUCTION

The conditions that cause the development of symptoms of frenching in tobacco (*Nicotiana tabacum* L.) plants are unknown. Low nitrogen,[3] low acidity, and excessive moisture[4] have at times been held to be contributing factors. Nevertheless soil from fields of severely frenched plants was often incapable of producing this morphological abnormality in the greenhouse. Abnormal plants transplanted to fresh soil almost always resume normal growth. The disorder is nontransmissible. Spencer[5] and others have therefore suggested that a labile organic substance (toxin) sometimes present in soils may be the causative agent in this disease.

Bacterial interrelations as revealed through studies with antibiotics seem to indicate the possibility that similar diffusates capable of a direct action on plants may also accumulate in soil under certain conditions. Data published by the writer[6 7] afford direct evidence that low concentrations of an organic substance, the amino acid *dl*-isoleucine, in contact with the roots of tobacco seedlings in aseptic cultures may bring about morphological abnormalities resembling those of frenching.

The present studies were therefore undertaken to determine whether any of the more common species of soil bacteria form diffusates capable of causing abnormal alterations in gross morphology of tobacco plants. The seedlings were aseptically grown in a constant environment. The growth medium selected was a slightly acid mineral agar containing a trace of Bacto-peptone. With or without sucrose

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[2] The writer wishes to express his appreciation for the courtesy shown him by N. R. Smith, Division of Soils, Fertilizers, and Irrigation, Bureau of Plant Industry, Soils, and Agricultural Engineering, who selected and furnished cultures of all the bacteria used.

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this medium was very favorable for growth of tobacco seedlings, but unfavorable for the development of the bacteria studied.

EXPERIMENTAL PROCEDURES

Seedlings of Xanthi Turkish tobacco were grown aseptically on 50 cc. of a mineral agar of pH 5.5 containing 200 p. p. m. of Bactopeptone in 200-cc. Erlenmeyer flasks at 25° C. in continuous light. Illumination of about 500 foot-candles was furnished by 3,500° white fluorescent lamps. Other details of the procedure have been given in a previous publication.[8]

Bacterial inoculation of aseptic cultures was made with a straight, sharpened needle, the stab being located ¾ to 1½ inches from the stem of the seedling. The age of the seedlings at the time of inoculation varied, the range being about 3 to 7 days.

GROWTH RESPONSES TO VARIOUS BACTERIAL SPECIES

The abnormalities in form of Xanthi Turkish tobacco seedlings grown in flasks inoculated with 27 species of bacteria are tabulated in table 1. Inoculations were made 1 inch from stems of seedlings in aseptic culture 1 week after the plant was inserted. Plants were harvested 3 weeks later. Of the 30 bacterial cultures 19 form nitrite from nitrate; 2 of the organisms, *Erwinia carotovora* and *Phytomonas tumefaciens*, are plant pathogens. Two strains each of *Agrobacterium radiobacter*, *Escherichia coli*, and *Pseudomonas fluorescens* are also included.

Abnormalities in growth of tobacco seedlings were caused by 10 of the 25 nonpathogenic species of bacteria in the absence of sucrose. *Erwinia carotovora* also brought about abnormalities in gross morphology. Symptoms with various species differed as a rule, but not always. Those with *Bacillus cereus* and *Erwinia carotovora* appeared identical. On the other hand, one strain of *Agrobacterium radiobacter* produced symptoms of injury in tobacco but the other did not. Symptoms included narrow, strap, cupped, rim-bound, and rim-rolled leaves and chlorosis and epinasty. Cupped and rim-bound leaves might be concave up or down. Chlorosis varied from general to reticular and gave some indication of association with the capacity for nitrite formation from nitrate by bacteria. Chlorosis did not occur as frequently with bacteria incapable of forming nitrite. The addition of 2 percent of sucrose to the medium led to a loss in "hormonoid" [9] action in 7 species of bacteria and a gain in 3 species. In no instance did the seedlings show any indication of root injury, nor were the bacterial colonies in actual contact with the roots.

[8] See footnote 7, p. 199.

[9] A hormone is defined as an organic compound formed by an organism that serves to regulate its cellular interrelations. The chemical compounds in the diffusates from these bacteria may or may not be identical in composition with those having a similar action in the tobacco plant. Pending proof of identity, the use of the term "hormonoid" (hormonelike) is suggested. The synthetic growth-regulating substances would fall into this category.

TABLE 1.—*Symptoms of injury shown by Xanthi Turkish tobacco seedlings grown in aseptic cultures inoculated with 25 nonpathogenic soil bacteria and 2 phytopathogenic bacteria*

[Seedlings grown for 3 to 7 days in aseptic cultures before they were inoculated; discarded when about 4 weeks old]

Culture No.	Organism		Nitrite produced [2]	Symptoms produced when	
	Name	Strain No. [1]		Sucrose absent	Sucrose present
1	*Aerobacter aerogenes* (Kruse) Beijer.	104-48	+	Reticular chlorosis	Reticular chlorosis.
2	*Aerobacter cloacae* (Jordan) Bergey et al.	108-98	+	White leaves; dwarfed plants.	
3	*Agrobacterium radiobacter* (Beijer. and Van Delden) Bergey et al.	96-433	0		
4	do	98-189	+	Leaf-tip chlorosis, dwarfed plants	
5	*Bacillus brevis* Mig	751	+		
6	*Bacillus cereus* Frankland and Frankland.	342	+	Reticulated (chlorosis) and scalloped strap leaves.	
7	*Bacillus circulans* Jordan	358	+		
8	*Bacillus megatherium* D By	343	0		
9	*Bacillus pumilus* Gottheil	272	0	Dark, narrow, down-cupped leaves with uprolled rims.	
10	*Bacillus Sphaericus* Neide	348	0		
11	*Bacillus subtilis* Cohn emend. Prazmowski.	231	+		Light-green, dwarfed plant.
12	*Bacterium denurificans* Lehmann and Neumann.	100-12	+		
13	*Bacterium globiform* Conn	168-110	?		
14	*Chromobacterium violaceum* (Schroet.) Bergonzini.	114-103	+		Very faint mottle.
15	*Corynebacterium simplex* Jens	140-47	?	Leaf epinasty, rim uproll, terminal bud killed	Leaf epinasty; down-cupped leaves.
16	*Corynebacterium tumescens* Jens.	141-45	?	Dark, narrow, down-cupped leaves with uprolled rims.	
17	*Erwinia carotovora* (L. R. Jones) Holland.	171-120	+	Reticulated (chlorosis) and scalloped strap leaves.	
18	*Escherichia coli* (Mig.) Castellani and Chalmers.	102-80	+	White leaves and bud; dwarfed plant.	
19	do	107-201	+	Reticular chlorosis	
20	*Micrococcus luteus* (Schroet.) Cohn	101-244	+		
21	*Phytomonas tumefaciens* (E. F. Sm. and Town.) Bergey et al.	172-102	?		
22	*Proteus vulgaris* Hauser	1354-44	+		
23	*Pseudomonas aeruginosa* (Schroet.) Mig.	110-98	+	White leaves and bud; dwarfed plant.	General chlorosis.
24	*Pseudomonas fluorescens* Mig	74-210	+		
25	do	112-277	+		White leaves and bud, dwarfed plant.
26	*Pseudomonas ovalis* Chester	77-180	0		
27	*Pseudomonas schuylkilliensis* Chester.	75-180	0		
28	*Sarcina flava* D By	68-178	0		
29	*Serratia lactorubefaciens* (Gruber) Bergey et al.	139-60	+		
30	*Serratia marcescens* Bizio	175-265	+	Reticular chlorosis	Mottled.

[1] Number of N. R. Smith, Division of Soils, Fertilizers, and Irrigations.
[2] Data furnished by N. R. Smith.

The types of morphological abnormalities resulting from diffusates from bacterial colonies are illustrated in figures 1 to 4. A frenching-like reticular chlorosis due to the presence of *Serratia marcescens* is shown in figure 1, *A;* epinasty associated with *Corynebacterium simplex* in figure 1, *B;* chlorosis associated with *Aerobacter aerogenes* and resembling that caused by iron deficiency in figure 1, *C.* The seedlings inoculated with *Aerobacter aerogenes* (fig. 2, *A*), *Corynebacterium simplex* (fig. 2, *B*), and *Pseudomonas aeruginosa* (fig. 2, *C*) illustrate symptoms obtained in the presence of sucrose, namely, yellow bud, epinasty, and a uniform, minus-sulfur type of chlorosis.

Figure 3 shows cultures inoculated with *Corynebacterium tumescens* (*A*), *Erwinia carotovora* (*B*), and *Bacillus cereus* (*C*), respectively. The first (*A*) shows epinasty and long, narrow leaves, whereas the symptoms with *E. carotovora* and *B. cereus* are almost identical in appearance. The last two show a well-defined, reticular chlorosis and narrow strap leaves with lobed or scalloped edges. The appearance of reticular chlorosis preceded that of strap leaves.

Cultures inoculated with *Bacillus pumilus* (No. 272) are shown in figure 4. No sucrose was added to the flask illustrated in *A*, but that in *B* contained 2 percent of sucrose. Inoculation took place after 3 days' growth of the seedlings and at the very margins of the agar. Epinasty, marginal leaf roll, narrow leaf, and cupped leaf are visible.

GROWTH RESPONSES TO VARIOUS STRAINS OF BACILLUS PUMILUS

Further tests were made with 29 strains of *Bacillus pumilus* to determine the range of symptoms to be expected in a single species. The symptoms obtained in these tests, which were made in the absence of sucrose, are listed in table 2. Eighteen of the strains led to no

TABLE 2.—*Variations in symptoms of abnormality in Xanthi Turkish tobacco with different strains of Bacillus pumilus after 28 days' growth* [1]

Culture No.	Strain No.	Symptoms of injury
1	A21	
2	A32	
3	A35	Epinasty; concave-up leaves with marginal uproll, short stem.
4	A170	Epinasty, narrow leaves with marginal uproll, tall stem.
5	A654	Dark, narrow, hooked leaves with marginal uproll and concave-down leaves.
6	A704	
7	236	Narrow, concave-down leaves with marginal uproll.
8	266	Narrow leaves with marginal uproll; tall stem.
9	307	Epinasty; concave-down leaves with marginal uproll.
10	331	Narrow leaves with marginal uproll; tall stems.
11	333	Epinasty; concave down leaves; short stem.
12	334	
13	345	Reticular chlorosis.
14	355	
15	576	
16	577	
17	620	
18	629	
19	630	
20	637	Leaves with marginal uproll.
21	657	
22	706	
23	707	
24	724	
25	725	
26	734	Retarded growth.
27	735	
28	788	
29	1034	

[1] Flasks inoculated on extreme periphery when seedlings were 3 days old.

FIGURE 1.—Seedlings of Xanthi Turkish tobacco in aseptic culture after inoculation of agar 1 inch from stems with nonpathogenic soil bacteria: *A*, *Serratia marcescens; B, Corynebacterium simplex; C, Aerobacter aerogenes.* Note the reticular chlorosis in *A*, the epinasty of leaves and death of terminal bud in *B*, and the chlorosis resembling that caused by iron deficiency in *C*.

FIGURE 2.—Seedlings of Xanthi Turkish tobacco in aseptic culture with 2 percent of sucrose after inoculation of agar 1 inch from stems with nonpathogenic soil bacteria: *A, Aerobacter aerogenes; B, Corynebacterium simplex; C, Pseudomonas aeruginosa.* Note yellow bud in *A*, cupped leaves with uprolled rims in *B*, and whiteness of plant in *C*.

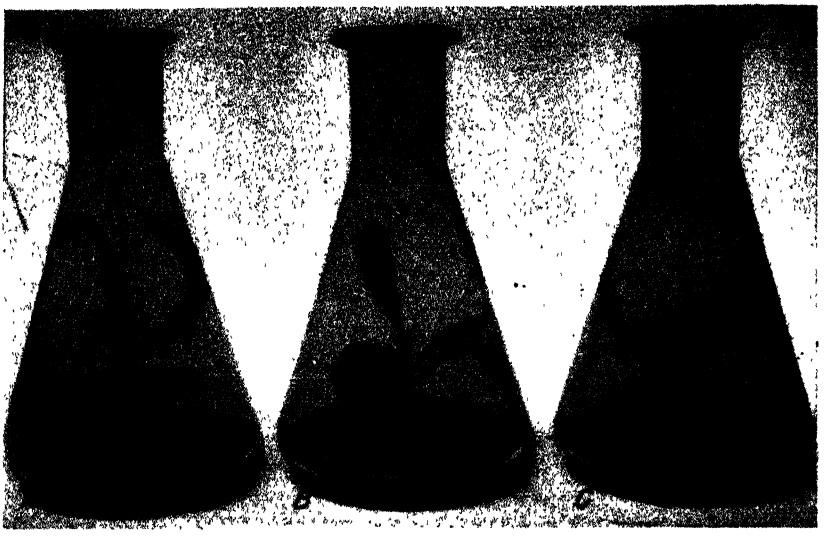

FIGURE 3.—Seedlings of Xanthi Turkish tobacco in aseptic culture after inoculation of agar at margin with various bacteria: *A*, *Corynebacterium tumescens; B*, *Erwinia carotovora; C*, *Bacillus cereus*. Note narrow leaves and epinasty in *A* and reticular chlorosis and scalloped strap leaves in *B* and *C*.

symptoms of abnormality in the seedlings. Some seedlings in inoculated flasks appeared even more vigorous than the controls. Symptoms associated with the other 11 strains ranged from simple retarded growth to epinasty, concave-up leaves with marginal uproll, and short stem. Intermediate abnormalities included these symptoms in varying proportion, or certain symptoms were absent. Reticular chlorosis only was associated with 1 strain and marginal uproll alone with another.

DISCUSSION

The number of nonpathogenic bacterial strains in soils is probably exceedingly great. A discussion of hormonoid actions brought about by their diffusates based on studies with less than 60 strains must therefore necessarily be limited in scope. The data suffice, however, to demonstrate in a conclusive manner that such hormonoid effects on tobacco can take place under appropriate conditions with bacterial species of general occurrence. Isolation and study of these compounds of bacterial activities may well prove of importance in many respects.

It was found in miscellaneous unreported experiments that the extent of the alterations in form of seedlings seemed to increase with nearness of the point of inoculation of the agar to the stem and the number of inoculations. Other known factors were size of seedling and the presence or absence of sucrose. Sometimes, however, severity of symptoms varied for no known reason, as in flasks inoculated with *Serratia marcescens*. Reproduction of symptoms occurred regularly with *Corynebacterium simplex* and *Bacillus pumilus*. The type of hormonoid action was not entirely specific for any one species, but

FIGURE 4.—Seedlings of Xanthi Turkish tobacco in aseptic culture after inoculation of agar at margin with *Bacillus pumilus* (No. 272): *A*, Without sucrose; *B*, with sucrose. Note epinasty and death of terminal bud in *A* and epinasty and narrow, hooked, rim-rolled leaves in *B*.

appeared to vary with the strain. In one instance *Erwinia carotovora* and *Bacillus cereus* produced identical symptoms.

Growth of the bacteria used was usually limited to the inoculation stab and did not appear to interfere with root growth. As a rule, bacterial growth was very scant and the colony was difficult to locate on the unfavorable medium employed. The roots in all cases appeared normal. In many instances morphological abnormalities appeared in the seedlings when no roots were in the immediate vicinity of the bacterial colony.

Proof of the existence of hormonoid responses to bacterial activity in the soil is regarded as an important consideration in elucidation of the cause of frenching in tobacco plants. The basic cause of this profound change in morphology in field plants is unknown, although the alteration is no greater than that effected by soluble metabolic products of some of the bacteria observed in aseptic culture. It might therefore be surmised that frenching is due to a similar action of metabolites from one or more bacterial forms. Hormonoid accumula-

tion in soil probably requires specific environmental conditions. As already mentioned [10] a close approximation of the symptoms of frenching can be obtained with *dl*-isoleucine.

SUMMARY

Xanthi Turkish tobacco seedlings were grown aseptically on 50 cc. of a mineral-agar solution containing 200 p. p. m. of Bacto-peptone in 200-cc. Erlenmeyer flasks at 25° C. with 500 foot-candles of continuous light. Inoculations (stab) of the agar at a distance from the stems of the seedlings with about 60 species and strains of presumably nonpathogenic soil bacteria led in several cases to alterations in gross morphology of the seedlings. These hormonoid effects included various types of chlorosis that simulated various mineral deficiencies, epinasty, cupped, narrow, and strap leaves, and leaves with lobes, hooked tips, and rim roll. Symptoms of abnormality were correlated with distance and number of bacterial colonies, age of seedlings, and bacterial strain. An analogy is drawn between the hormonoid effects of the diffusates from certain common soil bacteria and those obtained in frenching of tobacco.

[10] See footnote 7, p. 199.

USE OF LANOLIN AND OTHER UNGUENTS FOR IMPROVING BUDDING IN HEVEA RUBBERTREES [1]

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INTRODUCTION

In a program of top working mature trees of Para rubber (*Hevea brasiliensis* (H. B. K.) Muell. Arg.) at the United States Plant Introduction Garden, Coconut Grove, Fla., considerable difficulty was encountered in finding a satisfactory dressing for treating wounds made by the removal of large branches or of the entire heads of trees. Success reported for the use of lanolin [2] for the treatment of wounds of oaks led in 1945 to trials of the material on *Hevea* wounds. At this Garden *Hevea* is grown on the deeper areas of soil tentatively referred to as part of the Rockdale series.[3]

USE OF LANOLIN MIXTURE ON TREE WOUNDS

Direct application of lanolin to wounds on *Hevea* rubbertrees in full sun proved unsatisfactory as the lanolin quickly melted and ran down the treated branches or was greatly absorbed by the wood or the adjacent bark. To overcome this difficulty beeswax was added to the lanolin in equal parts. This combination was stiffer than lanolin and had to be applied with a brush after being melted in a grafting-wax pot. Even this mixture had a tendency to run on very hot, sunny days and to crack somewhat on cool days. An improvement was made by coating the wound with the lanolin-beeswax mixture and over this applying one or two thicknesses of cheesecloth, which were then covered with the mixture. The cheesecloth held the mixture in place, prevented cracking, and prolonged the sealing effect. The exposed wood of wounds treated in this manner did not become discolored, as the mixture apparently excluded attacking fungi, and cambial growth was much more rapid than on untreated wounds or on those treated with ordinary paint or several of the asphalt-base wound paints.

[1] Received for publication November 22, 1946.

[2] UNITED STATES FOREST SERVICE ALLEGHENY FOREST EXPERIMENT STATION. QUICK HEALING OF TREE WOUNDS. Allegheny Forest Expt. Sta. Ann. Rpt. 1943, pp. [24]-[25], illus. 1943. [Processed.] [Review in Horticulture 22: 293. 1944.]

[3] HENDERSON, J. R. THE SOILS OF FLORIDA. Fla. Agr. Expt. Sta. Bul. 334, 67 pp., illus. 1939. On p. 54 of this bulletin the Rockdale series is described as "oolitic limestone with numerous small surface cavities filled with red or reddish-brown sandy loams and silt loams or gray to grayish-brown sands to loamy sands."

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USE OF LANOLIN MIXTURE ON TOP-WORK BUDDINGS

The fact that lanolin protected or stimulated the cambium of wounds on rubbertrees and preserved the wood suggested the possibility that it might also be of benefit in budding operations in *Hevea* nurseries or in top working mature trees. In *Hevea* nurseries many budders have observed that high percentages of buds that seemingly were in excellent condition when unwrapped died during the succeeding 1- or 2-week interval before the tops of the stock plants above them were cut off to force the dormant buds into growth. Experience in

FIGURE 1.—*A*, Untreated budding of *Hevea* rubbertree, 8 days after being unwrapped. Note that the exposed cambium surrounding the bud patch is dead and the wood weathered and discolored; although the bud patch is in good condition, it was dead when examined 12 days later. *B*, Bud patch and panel, 8 days after being unwrapped and treated with 3 : 1 lanolin-beeswax mixture. Note that the bud patch is in good condition and that the surrounding cambium on the panel has survived. × about 1.

the American Tropics has shown that such losses may be as high as 75 percent.

As a preliminary step in determining the effect of lanolin on bud survival after unwrapping, several buds were top worked in large trees. On opening, half of the bud patches and the surrounding exposed cambium on the stocks were smeared with a mixture of 3 parts of lanolin and 1 part of beeswax, which was soft enough at air temperature for application with a finger. The other buds in the test were left untreated. During almost daily examinations that followed the unwrapping of the buds it was observed that when the buds were treated there was no discoloration of the cambial tissue surrounding the patch, which remained green, whereas the exposed cambium surrounding untreated patches began to darken within a few hours and became increasingly darker for several days. In some cases this tissue became spotted with mold. These conditions also prevailed in later buddings on *Hevea* seedlings (fig. 1). During this test it also was noted that the cambium around the treated buds grew so rapidly that it tended to grow over the edges of the patches 4 or 5 weeks after opening. Had the buds been allowed to remain dormant an equal number of months, no doubt some of them would have been completely overgrown.

USE OF LANOLIN MIXTURE ON BUDDINGS IN NURSERIES

The test of the lanolin-beeswax mixture on top-work buddings was not large enough to give conclusive proof, but it did show a definite advantage for its use on opened buds and led to the setting up of a more extensive experiment. One hundred seedlings were budded in the usual manner on May 22, 1945, and were unwrapped and examined 15 days later, on June 6, when it was found that all buddings had been successful and the bud patches were in excellent condition. As the buds were opened and examined, every other one was coated with a smear of 3 : 1 lanolin-beeswax mixture. Thus, there were 50 treated and 50 untreated buds. Examination of these buds on June 12 showed that 8 untreated buds were dead and all 50 of the treated ones were in excellent condition. On June 17 the treated buds were still in good condition, but 16 untreated ones were dead. Final examination was made on June 26, 20 days after opening, when 3 treated buds and a total of 18 untreated ones were dead. Thus, 94 percent of the treated buds remained alive in contrast to but 64 percent of those untreated.

Observations during this test suggested that some improvement might be obtained by applying the lanolin mixture at budding time in the fissure formed by the bulge of the flap covering the bud after its insertion. Wrapping of the bud with waxed tape was expected to force the lanolin under the flap and afford a protective covering to the exposed cambium of the stock surrounding the bud.

On June 12, 100 seedlings were budded; 50 of these were treated by the method just explained with the 3 : 1 lanolin-beeswax mixture and 50 were left untreated. When unwrapped 14 days later, all buds were in excellent condition. Of the 50 buds treated at budding, 25 were smeared again with the lanolin-beeswax mixture immediately on opening; the remaining 25 were not treated a second time. Of the 50

buds not treated at budding, 25 were smeared with the mixture on opening and the remaining 25 were left untreated as checks.

All treated buds, whether treated at budding, opening, or both, were in good condition when examined 8 days after unwrapping, whereas 6 of the untreated checks were dead. Final examination of these buds was made on July 31, 35 days after opening, when 5 of the buds treated at budding and again at opening and 5 of those treated only at budding were dead; 9 untreated check buds were lost, as compared with none for buds treated only at opening. When treated at budding apparently there was some forcing of the lanolin-beeswax mixture under the bud patch by the pressure exerted by the wrapping tape. From observations in subsequent tests, which gave the same indication, treatment at budding seldom equaled treatment at opening only as a means of reducing loss in *Hevea* buddings.

USE OF VARIOUS UNGUENTS ON BUDS

In order to observe the effects of applying unguents other than the lanolin-beeswax mixture to the bud-patch area, seedlings were treated at the time of budding as follows: 10 with a 3 : 1 vaseline-beeswax mixture, 10 with a 3 : 1 vaseline-paraffin mixture, 10 with ordinary automobile cup grease, 10 with a 3 : 1 lanolin-beeswax mixture, and 10 left untreated as checks. These buds were unwrapped after 14 days, when they were again treated with the same combinations. On opening, all buds with the exception of 1 that was treated with cup grease were found in good condition. Final examination 24 days after this opening showed that 7 check buds, 1 treated with the vaseline-beeswax mixture, 2 with the lanolin-beeswax mixture, 9 with cup grease, and 1 with the vaseline-paraffin mixture were dead. The more damaging effect of cup grease was attributed to its soft consistency and the greater ease with which it was forced under the bud patch when pressure was applied with the budding tape.

To determine the effect of these same materials when applied on buds at the time of opening and not at the time of budding, 85 additional seedlings were budded. When unwrapped in 14 days, all were found to be in good condition and all were immediately treated with the same materials as used in the preceding test; there were 17 buds in each of 5 treatments. Final examination of the buds, 25 days after opening, showed that 7 untreated checks and 1 treated with cup grease were dead. All buds treated with lanolin-beeswax, vaseline-beeswax, and vaseline-paraffin mixtures were in perfect condition. From this it appears that any one of many inexpensive nontoxic materials of the proper consistency possibly might be applied with equal success as a protective coating over the exposed cambial tissue upon unwrapping the bud, but with the limited stock material at the disposal of the writers more extended tests were impossible.

USE OF LANOLIN MIXTURE ON BUDS FROM STORED BUDWOOD

As the foregoing experiments were conducted with freshly cut budwood, it was considered advisable to test the treatments on buds taken from budwood that had been stored for various periods under conditions approaching those encountered during long-distance shipments.

A quantity of budwood was cut on June 27 and divided into 2 equal lots. Each lot was carefully packed in slightly damp sphagnum moss and wrapped in kraft paper. Both packages were stored indoors at air temperature. After 5 days 1 of the packages was opened and from the budwood 50 seedlings were budded. Half of these were treated with 3:1 lanolin-beeswax mixture at budding, and the other half were left untreated as checks. When the buds were opened 14 days later, all were found to be in excellent condition and the treated ones were given a second application of the mixture. Twenty-four days later it was found that only 3 of the treated buds had died in contrast to 8 of the check ones.

The second package of budwood was opened after a 10-day storage period and used in budding 50 seedlings which were treated in the same manner as those in the 5-day storage test. When opened after 14 days, 2 of the 25 check buds were dead but all the treated ones were in good condition. Twenty-four days after opening, a total of 11 of the original 25 check buds and 9 of the 25 treated with the lanolin-beeswax mixture at budding were dead.

At the time these two tests were begun the relation of treating at budding to treating only at opening had not been established, but it was apparent before their completion. Accordingly, another storage test was begun for the inclusion of both these types of treatment and the storage periods were increased to 10 and 20 days, respectively, as 5-day storage had appeared to have no definite aging effect on the budwood.

After the budwood had been stored 10 days, buds were inserted in 84 seedlings and every third bud was treated with the 3:1 lanolin-beeswax mixture. The remaining 56 buds were divided alternately into 2 groups of 28 buds each, 1 group being marked for treatment at opening and the other group for leaving untreated throughout the experiment as checks. Upon unwrapping, 14 days after budding, 1 check bud, 2 buds to be treated at opening, and 1 bud treated at budding were dead. Immediately after the unwrapping of these buds the 27 living originally treated were treated again. Through an error 25 instead of the 26 living buds to be treated at opening were smeared with the lanolin-beeswax mixture. This automatically added 1 bud to the untreated check group.

Of the 28 check buds alive at opening 17 (60.7 percent) were in good condition at the final examination, 24 days after unwrapping; of the 25 buds alive at opening and treated with lanolin at that time 21 (84.0 percent) were in good condition; of the 27 buds treated twice with lanolin and alive at opening 21 (77.8 percent) survived.

From budwood that had been stored 20 days 60 seedlings were budded. Through an oversight an unequal division was made of the seedlings between treatments, as 20 buds were treated with 3 : 1 lanolin-beeswax mixture at budding, 18 were held for treatment at opening, and 22 were maintained as untreated checks. When unwrapped 14 days later, 18 of the treated buds, 15 of those held for treatment at opening, and 19 of the check buds were alive. The 18 buds treated at budding were treated a second time at opening. When final examination of the buds alive at opening was made 24 days later, it was found that 13 (72.2 percent) of those treated at budding and again at opening were alive; 11 (73.3 percent) treated

only at opening were alive; and only 11 (57.9 percent) of the checks had survived.

EFFECT OF TIME OF TOPPING STOCKS ON BUDS TREATED WITH LANOLIN MIXTURE

As a final experiment in the use of lanolin as a means of reducing loss in *Hevea* buddings 100 additional seedlings were budded on August 2. These were unwrapped after 14 days, and all were found to be in good condition. At opening, 50 were smeared with the 3:1 lanolin-beeswax mixture and 50 were not treated. Stocks of half of each group of 50 were immediately cut off in the usual manner to force bud growth and the cut surface of the stock was painted with the 1:1 lanolin-beeswax wound mixture. Thus, 50 stocks into which 25 check buds and 25 treated buds had been inserted were cut back, but the remaining 50 were not cut. All uncut stock plants with living buds were cut back 12 days after opening to force the buds. By this time 11 of the previously uncut checks, 7 cut checks, and 1 cut lanolin-treated bud had died. Final examination was made 25 days after opening, when 11 of the checks not cut back at opening, 9 cut checks, 3 uncut lanolin-treated buds, and 1 cut lanolin-treated bud were dead. The results of this limited experiment indicate that seedlings containing living bud patches may, when unwrapped, be cut back immediately to force sprouting without fear of serious loss if the patches and surrounding cambium are treated with lanolin-beeswax mixture at the same time. In this and other experiments where a delay of 10 or more days occurred between the unwrapping of the bud and the cutting back of the stock, survival of bud patches treated with the lanolin-beeswax mixture at opening was greater than that of untreated buds.

EFFECT OF LANOLIN MIXTURE ON SPROUTING OF BUDS

An observation in connection with the immediately preceding experiment and one other involving the cutting back of stocks to force bud growth was the retarding effect the application of the 3:1 lanolin-beeswax mixture had on the growth of the buds.

In the experiment of May 22 in which 50 buds were treated with the 3:1 lanolin-beeswax mixture at opening and 50 were left untreated as checks, all living buds had the stocks above them cut back 11 days after opening to force the buds. Nineteen days after the cutting back of the stock 37 of the 47 surviving lanolin-treated buds were either swelling or sprouting, but only 1 had produced a shoot more than 1 mm. long; 26 of the 32 check buds were either swelling or sprouting, and 8 of these had shoots more than 1 mm. long. Final examination, 34 days after the cutting back of the stocks, showed all lanolin-treated and all check buds to be swelling or to have shoots. All but 1 of the check buds had shoots with an average length of 282.4 mm., whereas only 34 of the 47 lanolin-treated buds had produced shoots and these had an average length of only 91.4 mm.

The same retarding effect was observed in the experiment begun on August 2 in which 50 stocks were topped as soon as the buds were unwrapped and the remaining 50 were cut back 12 days after un-

wrapping. As reported for that experiment, half of the buds in each group had been treated with the 3:1 lanolin-beeswax mixture at opening whereas the others were left untreated as checks. In the seedlings cut back when the buds were opened, 4 of the 18 living untreated check buds were sprouting 12 days later when the other seedlings were topped, while only 3 of the 24 buds treated with lanolin mixture were sprouting.

At the end of this experiment, 25 days after the buds were unwrapped, 14 (87.5 percent) of the 16 living check buds in stocks cut back at opening were sprouting and 14 (58.3 percent) of the 24 living lanolin-treated buds in correspondingly cut-back stocks had sprouted. In the seedlings cut back 12 days after the buds were unwrapped 7 (50 percent) of the 14 living check buds had sprouted, whereas only 7 (31.8 percent) of the 22 living lanolin-treated ones had done so.

SUMMARY

Wounds arising from the pruning of *Hevea* rubbertrees kept in better condition and healed more rapidly when treated with 1:1 lanolin-beeswax mixture than when treated with any other wound dressing.

Buds from fresh budwood or from that stored for periods up to 20 days, when set in the tops of mature trees or in the customary location in seedlings, survived in much greater numbers when the bud patch and the surrounding area of cambium on the stock were treated with 3:1 lanolin-beeswax mixture at opening, or unwrapping, than did untreated buds.

Single treatment with the 3:1 lanolin-beeswax mixture at time of budding or treatment at time of budding and again at opening seldom was found to be as beneficial as treating only at the time of unwrapping the buds. Several other unguents that gave material benefit when applied at opening of the buds were used, but the softest of these caused increased mortality to buds when applied at time of budding.

Applications of lanolin-beeswax mixtures to bud patches and surrounding cambium and to the cut surface of the stock may allow cutting back of the stock at the time of unwrapping of the buds with negligible loss, thus eliminating the usual waiting period of 10 to 14 days between opening of the buds and cutting back of the stock during which under present practice the death or survival of the buds is determined.

Buds treated with the 3:1 lanolin-beeswax mixture were found to sprout more slowly after cutting back of the stocks than did untreated ones.

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EFFECTS OF CONTROLLED SOIL MOISTURE ON GROWTH, COMPOSITION, YIELD, AND QUALITY OF MARYLAND TOBACCO [1]

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INTRODUCTION

Adequate soil and air moisture constitutes one of the cardinal requirements for the growth of leaf tobacco which must meet exacting commercial demands. Since the leaf of the tobacco plant (*Nicotiana tabacum* L.) is the final product, it must possess a more or less definite combination of shape, size, structure, elasticity, venation, color, and possibly other structural details not readily visible to the eye, as well as a rather characteristic chemical composition which determines aroma, taste, and unidentified characters indicative of quality. The fire-holding capacity of the cured leaf is strikingly modified by both the structural details and the chemical composition. These properties in turn are influenced and modified by soil moisture, which largely controls the growth of all parts of the plant, especially the leaf structure.

It is generally recognized by the tobacco trade that leaf grown during a dry season is very different from that grown during a wet one. The adaptability of the two products to manufacturing purposes differs widely. The leaf produced during a dry season is small, dark and dull in color, high in nicotine, and lacking in elasticity. It possesses more aroma associated with gums and resins, has a dense structure associated with a high weight per unit area, characteristically possesses low fire-holding capacity, and manifests a slow and inactive fermentation when bulked or packed. The cured leaf from tobacco produced during a season with adequate and well-distributed moisture, on the other hand, other conditions being equal, is thin, of an open structure or texture associated with a light weight per unit area, comparatively large, light and bright in color, elastic, low in nicotine, weak in aroma, and low in gums and resins and shows an active and rapid fermentation when bulked.

Although to a greater or less extent soil moisture appears to be the dominant factor, atmospheric humidity, shading associated with cloudiness, and air movements that increase the evaporating power of the air are all a part of the complex which controls plant growth and development. The organic matter and the mineral constituents that affect the physical condition of the soil and control the retention

[1] Received for publication December 23, 1946. Cooperative investigations by the Division of Tobacco, Medicinal, and Special Crops and the Maryland Agricultural Experiment Station.

and release of moisture to the growing plant are highly important factors in the utilization of soil moisture by plants in the development of leaves.

In the 1941 Yearbook of the United States Department of Agriculture Garner (*5*)[2] discussed the effects of climate on the growth of tobacco. There are few publications dealing with the effects of supplemental water on the production of tobacco in humid areas. The work by Goff (*7*) was possibly the earliest account in this country of the effect of irrigation on the growth of tobacco. Anderson and his associates (*1*, *2*, *3*) reported the effects of irrigation on the yield and quality of cigar tobacco at the Tobacco and Vegetable Substation at Windsor, Conn.

The classic work of Hasselbring (*9*, *10*), although it does not deal directly with irrigation, has a bearing on the subject since shading affects plant and soil-moisture relations. This relation was further emphasized by the work of Stewart (*16*), who showed that average soil moisture, air temperature, and humidity were higher when tobacco was grown under a cloth shade than when grown under comparable conditions without shade. A partial explanation of some of these effects may be found in the work of G. W. Volk (*17*) and N. J. Volk (*18*), who studied the effects of the wetting and drying of a soil on potash fixation. McMurtrey (*13*) described the symptoms of nitrogen deficiency, which, according to Anderson and his coworkers (*1*, *2*, *3*), are often associated with irrigation effects. The review by Kramer (*12*) brought up to date the contributions on soil moisture in relation to plant growth.

MATERIALS AND METHODS

As previously stated, tobacco leaf grown during dry weather manifests certain characteristics that differ from those of leaf produced during wet weather. Since the two conditions do not occur simultaneously, a direct comparison of the leaf produced in the same season under the two conditions is not possible. It seemed desirable, therefore, to set up experimentally these two conditions insofar as possible in order to arrive at a more complete understanding of the effects of insufficient and of adequate moisture on growth and character of the leaf produced. Such effects were evaluated by systematic measurements, chemical analyses, and any other evident changes in the character of the leaf.

The unvaried cultural and handling procedures (*15*) in general were those used by successful growers of the Maryland type of tobacco. Crop yields and values were determined after stripping, grading, weighing, and sampling. Samples so obtained were submitted to experienced judges of Maryland tobacco, who assigned values. Calculations based upon weights and values thus obtained were the source of the results reported on yields and gross values per acre. Tests to determine the fire-holding capacity of cured leaves of the cigarette grades were made according to the usual laboratory procedures. Twenty-five leaves from as many plants were ignited by an electric lighter. These tests were carried out under controlled temperature and humidity.

[2] Italic numbers in parentheses refer to Literature Cited, p. 248.

The various artificial weather conditions were set up after the plants had become well established. There were three treatments in 1934, each on a separate plot, which was given the same number as its treatment. One area was held dry continuously by means of a tarpaulin rolled down just before a rain and supported by suitable scaffolding, as shown in figure 1. The second area was irrigated twice weekly, receiving one-fourth inch of water each time during weeks when no rain fell, and the third area in like manner received an equivalent of one-half inch of water. The water was added as a spray by means of overhead nozzles.

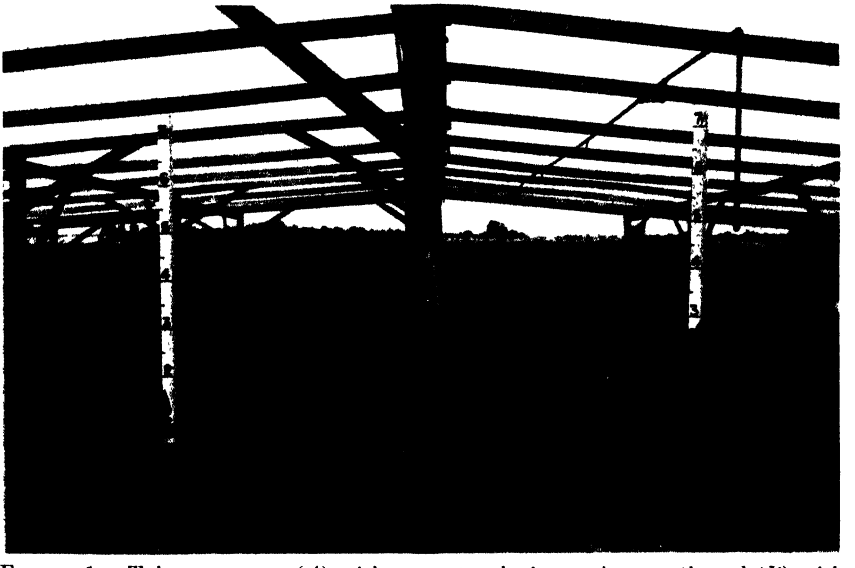

FIGURE 1.—Tobacco grown (*A*) without water during early growth and (*B*) with both rainfall and supplementary water equivalent to one-half inch twice a week when insufficient rain fell during early growth. Photographed August 10, 1936. Note scaffolding for support of canvas and movable irrigation pipe with nozzles.

In 1935 and thereafter five treatments were used on as many areas in two different series: A, lowland, and B, upland. In addition to the area kept dry continuously as in the previous year (No. 1), there was a second area irrigated by overhead nozzles to add one-half inch of water twice weekly if there was no rainfall during the week (treated like No. 3 in 1934). A third area was irrigated in the same manner early in the season and kept dry for the remainder of the season by means of a tarpaulin as on area 1. A fourth area was kept dry like area 1 during the early part of the season and irrigated like area 2 during the last part of the season. Tobacco grown on the fifth area (the control area) was produced under the prevailing rainfall. The plan was to roll the covers down just before a rain and to roll them up as soon thereafter as practicable. This procedure was followed in an effort to avoid shading. To give protection from showers during the night also, an attendant was on hand to apply covers when a shower threatened.

TABLE 1.—*Amounts of rainfall and irrigation water (inches) and dates of transplanting, replanting, irrigating, topping, and harvesting tobacco nd of applying and changing covers, Upper Marlboro, Md., 1934–40*

[C, Covers applied to withhold rainfall from plots 1A, 1B, 4A, and 4B; CR, covers changed from plot 4A to plot 3A and from plot 4B to plot 3B; W, water (equivalent to ½ inch) added to plots 2A, 2B, 3A, and 3B early in season and to plots 4A and 4B late in season; P, transplanting; RP, replanting; H, harvesting, T, topping. In the treatment or plot numbers, A indicates lowland series and B upland series. Zeros were not added to indicate that no rain fell on the dates when operations were carried on]

RAINFALL

Date	June							July							August							September [1]					4-month total						
	1934	1935	1936	1937	1938	1939	1940	1934	1935	1936	1937	1938	1939	1940	1934	1935	1936	1937	1938	1939	1940	1934	1935	1937	1939	1940	1934	1935	1936	1937	1938	1939	1940
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	37	0.01	0	0	0	0	0	0	0	0	0	W	1.63						
2	0	0	0	.07	0	0	0	0	0	.07	08	.10	0	0	0	W	0	.02	.07	W	W	0	0	0	0	0							
3	0	.04	.17	.37	0	0	0	0	0	{C .67}	C	0	1.45	W	0	0	W	{W .33}	.14	0	.56	.13	H	0	0								
4	0	0	0	0	0	0	0	0	0	.12	0	0	.08	46	0	0	0	0	.63	.08	0	0	0	2.65	0								
5	0	1.07	0	0	0	0	0	.57	0	0	2.50	0	.09	0	.25	0	03	0	0	0	0	0	3.88	0	0								
6	0	0	0	.07	0	0	0	0	0	0	0	0	0	0	0	W	0	W	0	0	{W .40}	0	{3.56 H}	0	H								
7	0	P	.01	.10	0	0	0	0	0	0	0	0	0	0	W	.80	25	0	0	0	.12	3.78		0									
8	0	.80	0	0	0	0	.12	.02	1.70	0	0	C	C	0	.03	0	0	.65	0	W	0	H		0									
9	0	0	0	0	0	0	.21	0	{C .48}	0	.05	.03	0	0	03	0	0	.06	W	0	W			0									
10	0	0	0	1.10	0	0	0	W	.61	{.15 W}	0	0	0	C	W	0	0	0	.17	0	0			85									
11	0	0	.64	RP	0	0	0	C	0	0	.67	0	W	0	0	0	.01	0	0	W	.19			0									
12	.21	.35	P	0	.29	0	0	0	0	.17	0	.06	0	1.45	.50	0	CR	{T CR 1.03}	{CR W}	0	0			H									
13	0	0	0	0	.01	1.57	0	.07	.80	0	0	0	.03	.55	0	{W .24}		.41	0	.03	W												
14	0	0	.13	1.17	0	0	0	0	0	W	.02	.10	{02 W}	0	0	.01	W	0	0	.02	0												
15	0	0	0	.25	P	0	0	0	0	0	.48	W	0	0	.04	0	0	0	0	W	{.48 T}												
16	0	0	0	.13	0	0	0	W	0	0	.08	0	0	.10	.76	CR	.07	0	0	0	0												
17	0	0	0	3.14	.25	0	{P .04}	0	0	W	0	1.48	0	0	0	0	0	W	.44	0	.03												
18	1.13	0	0	52	0	0	0	0	0	0	0	0	.21	0	0	0	W	0	0	{W 2.74}	0												
19	P	.91	0	0	31	.15	0	0	W	0	0	0	0	W	0	.06	0	0	0	0	.13												
20	0	0	0	0	11	0	0	W	0	0	.80	.20	0	0	0	.06	0	W	0	.05	{W CR}												

21	0	.18	0	0	.06	P	0	0	0	W	0	.64	.67	0	W	.80	1.35	.10	0	0	0	---	---	---	---	---	---	---	---	---	---	---	---
22	.05	.20	0	0	0	.50	0	0	0	0	0	.35	0	0	0	.17	0	0	0	0	0	---	---	---	---	---	---	---	---	---	---	---	---
23	0	0	0	0	.27	0	0	0	W	0	W	1.48	0	1.03	0	W	1.11	.84	W	0	W	---	---	---	---	---	---	---	---	---	---	---	---
24	0	0	.50	0	0	0	.04	W	.33	.89	0	0	0	.01	{W .76}	0	0	0	0	.50	0	---	---	---	---	---	---	---	---	---	---	---	---
25	0	0	0	0	0	0	0	.21	.08	0	0	.04	.10	0	.33	0	.07	2.19	H	1.51	0	---	---	---	---	---	---	---	---	---	---	---	---
26	0	0	0	0	0	0	0	0	0	.78	0	0	0	W	0	0	H	1.70	---	{.34 CR T}	1.31	---	---	---	---	---	---	---	---	---	---	---	---
27	.10	0	0	.57	.15	0	0	W	0	.56	W	0	.45	0	0	0	---	0	---		.50	---	---	---	---	---	---	---	---	---	---	---	---
28	0	0	0	0	3.15	.31	.47	0	0	0	0	0	0	.03	T	.03	---	0	---	.10	.63	---	---	---	---	---	---	---	---	---	---	---	---
29	0	0	0	.24	0	.75	0	1.29	0	.30	0	1.20	.38	0	0	T	---	0	---	.04	0	---	---	---	---	---	---	---	---	---	---	---	---
30	.61	0	.87	.66	0	.77	0	0	W	0	W	0	0	W	0	0	---	.30	---	.06	0	---	---	---	---	---	---	---	---	---	---	---	---
31	---	---	---	---	---	---	---	.02	0	0	.08	.08	.02	0	0	0	---	0	---	0	0	---	---	---	---	---	---	---	---	---	---	---	---
Total	2.10	3.55	2.32	8.39	4.60	4.05	.88	2.18	4.00	3.71	4.76	5.76	2.05	5.45	2.71	2.17	2.89	7.30	1.64	5.61	3.79	4.34	[3] 4.02	0	3.50	1.63	11.33	[1] 13.74	8.92	20.45	12.00	15.21	11.75

TOTAL IRRIGATION WATER APPLIED

Treatment No.																																	
1	0	0	0	0	0	0	0	0.25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.25	0	0	0	0	0	0
2	0	0	0	0	0	0	0	1.25	1.50	2.00	1.50	.50	1.00	1.50	1.25	2.00	1.00	2.00	2.00	2.50	3.00	0	0	0	.50	0	2.50	3.50	3.00	3.50	2.50	4.00	4.50
3	0	0	0	0	0	0	0	2.50	1.50	2.00	1.50	.50	1.00	1.50	2.50	1.50	0	1.00	1.00	2.50	2.50	0	0	0	0	0	5.00	3.00	2.00	2.50	1.50	3.50	4.00
4	---	0	0	0	0	0	0	---	0	0	0	0	0	0	---	.50	1.00	1.00	1.00	0	.50	---	0	0	.50	0	---	.50	1.00	1.00	1.00	.50	.50
5	---	0	0	0	0	0	0	---	0	0	0	0	0	0	---	0	0	0	0	0	0	---	0	0	0	0	---	0	0	0	0	0	0

TOTAL WATER (RAINFALL AND IRRIGATION)

Treatment No.																																	
1	2.10	3.55	2.32	8.39	4.60	4.05	0.88	0.84	1.70	0.07	0.08	0.10	0.17	2.28	0	0	0	0	0	0	0	0	0	0	0	0	2.94	5.25	2.39	8.47	4.70	4.22	3.16
2	2.10	3.55	2.32	8.39	4.60	4.05	.88	3.43	5.50	5.71	6.26	6.26	3.05	6.95	3.96	4.17	3.89	9.30	3.64	8.11	6.79	4.34	[2] 4.02	0	4.00	1.63	13.83	17.24	11.92	23.95	14.50	19.21	16.25
3	2.10	3.55	2.32	8.39	4.60	4.05	.88	4.68	5.50	5.71	6.26	6.26	3.05	6.95	5.21	2.55	.29	1.73	2.20	7.91	3.85	4.34	0	0	0	0	16.33	11.60	8.32	16.38	13.06	15.01	11.68
4	---	3.55	2.32	8.39	4.60	4.05	.88	---	1.70	.07	.08	.10	.17	2.28	---	1.62	3.60	7.57	1.44	.20	2.94	---	[2] 4.02	0	4.00	1.63	---	10.89	5.99	16.04	6.14	8.42	7.73
5	---	3.55	2.32	8.39	4.60	4.05	.88	---	4.00	3.71	4.76	5.76	2.05	5.45	---	2.17	2.89	7.30	1.64	5.61	3.79	---	[2] 4.02	0	3.50	1.63	---	13.74	8.92	20.45	12.00	15.21	11.75

[1] No data obtained for 1936 and 1938, because crop had been harvested (H).
[2] Planted May 26
[3] Harvest rainfall not included in total.

The rows were spaced 34 inches apart, and the plants were spaced the same distance in the rows. Each area was occupied by 8 rows of 14 plants each. The border plants were removed at harvest time; that is, the yields and other data were taken on the 72 plants inside the border. Each area received a fertilizer containing 4 percent of nitrogen (N) derived one-fourth from tankage, three-eighths from ammonium sulfate, and three-eighths from nitrate of soda; 8 percent of phosphoric acid (P_2O_5) derived from superphosphate; and 12 percent of potash (K_2O) derived from sulfate of potash. This mixture was applied in the row at the rate of 750 pounds per acre.

The dates of transplanting, watering, applying covers, changing covers, topping, harvesting, and replanting, as well as the dates and amount of rainfall and the amount of irrigation, are shown in table 1. Watering, or irrigation, was accomplished by means of overhead nozzles to simulate natural rainfall. Occasionally there was rain immediately after irrigation; this wet the soil excessively and produced more or less leaching on the irrigated areas. At times it was difficult to irrigate during windy periods because the spray tended to blow, but blowing was controlled satisfactorily by avoiding windy periods and by tilting the spray nozzles in such a way as to direct the spray to the desired area. The covered areas were left unprotected at the sides to avoid setting up a humid atmosphere under the tarpaulin, but no serious difficulty was encountered from blowing rains. Recording instruments under the tarpaulin showed no significant differences in temperatures and humidity from those recorded on an adjacent uncovered control area. It should be recognized, however, that the treatment on the dry plot did not entirely simulate natural drought conditions, because the atmosphere and the soil may not have been dry simultaneously. Besides, there were some effects of shading for it was not always desirable to roll up the tarpaulin immediately after a shower because of the danger of its molding and decaying.

The plots were located on soils of the Collington series, but the plants were not grown on the same areas for the entire period, because of the development of nematodes (*Heterodera marioni* (Cornu) Goodey) and of fusarium wilt, caused by *Fusarium oxysporum* f. *nicotianae* (J. Johnson) Snyder and Hansen. The lowland series (A) was located on one area in 1934, another in 1935, and a third from 1936 to 1940; on all of these the soil was mapped as Collington loamy sand and was definitely sandy with little or no subsoil. Except in 1936 and 1937, the upland series (B) was located on a slightly heavier soil with a definite subsoil at about 16 to 22 inches; it was mapped as Collington fine sandy loam. In 1936 and 1937 the soil was a loamy sand like that on which series A was located. The plots were in four different locations—one in 1934, a second in 1935, a third in 1936 and 1937, and a fourth from 1938 to 1940. The water applied to plots in the lowlands series was obtained from a deep well, and that applied to the upland series was ground water from a surface spring.

The Maryland Broadleaf variety was planted from 1934 to 1938 and the Maryland Medium Broadleaf in 1939 and 1940.

Some preliminary studies were made during 1934 and 1935 to determine the effect of irrigation on the percentage of moisture in the soil on a moist-weight basis (table 2). The soil samples were taken to a depth of 6 inches and represented 12 borings per plot. Soil

TABLE 2.—*Soil-moisture content based on moist weight before and after watering irrigation plots, Upper Marlboro, Md., 1934–35*

[Samples represent 12 borings per plot to a depth of 6 inches]

Date and treatment No.	Lowland (series A)		Upland (series B)	
	Before watering	After watering	Before watering	After watering
1934	*Percent*	*Percent*	*Percent*	*Percent*
June 27:				
1	4.37		6.76	
2	4.57		7.73	
3	6.47		9.92	
July 5:				
1	4.39		6.75	
2	4.76		7.83	
3	5.92		8.91	
July 10 and 11.				
1	4.37	6.39	6.23	6.13
2	4.75	6.34	6.92	9.11
3	6.23	6.38	8.61	13.38
July 16 and 17:				
1	4.21	4.37	5.40	5.71
2	4.67	6.12	7.00	9.35
3	7.06	7.89	9.48	12.13
July 24 and 25:				
1	3.46	4.06	5.19	5.49
2	4.30	5.48	5.98	7.46
3	5.91	8.27	8.81	11.57
July 31 and Aug. 1				
1	3.73	3.47	5.90	5.21
2	7.21	6.05	10.39	8.45
3	8.79	7.51	13.32	11.94
Aug. 7 and 8				
1	2.94	2.58	4.49	4.31
2	5.09	6.77	6.80	8.16
3	7.23	8.67	10.81	13.19
Aug. 14 and 15.				
1	2.88	2.57	3.71	3.76
2	7.29	6.28	9.65	7.80
3	9.27	9.21	13.93	12.93
Aug. 21 and 22:				
1	2.57	2.28	2.99	2.47
2	4.59	6.31	6.31	7.59
3	5.97	7.99	11.51	13.44
Aug. 28 and 29.				
1	1.85	1.90	2.75	2.81
2	6.74	5.30	8.32	7.67
3	7.70	6.95	13.18	12.92
Sept. 4 and 5:				
1	1.40	1.25	2.10	2.20
2	7.67	5.78	9.78	8.20
3	8.42	7.11	13.41	11.83
1935				
June 26.				
1	5.46		8.30	
2	5.23		8.13	
3	4.63		8.21	
4	5.43		7.83	
5	5.71		7.80	

Date and treatment No.	Lowland (series A)		Upland (series B)	
	Before watering	After watering	Before watering	After watering
1935	*Percent*	*Percent*	*Percent*	*Percent*
July 3:				
1	4.71		6.69	
2	4.62		6.21	
3	3.86		6.27	
4	4.24		6.37	
5	4.84		6.50	
July 9:				
1	4.72		6.38	
2	7.96		11.07	
3	7.71		10.99	
4	4.05		6.03	
5	8.26		10.88	
July 16.				
1	4.43		5.77	
2	5.62		10.17	
3	5.32		9.88	
4	4.61		6.14	
5	5.95		9.57	
July 23 and 24.				
1	3.45	3.64	4.42	4.20
2	4.94	8.57	7.66	10.61
3	3.90	8.11	8.29	11.13
4	2.65	2.48	5.01	4.82
5	4.16	6.91	6.61	8.79
July 30 and 31				
1	2.44	2.42	3.25	3.08
2	4.02	8.34	7.91	9.96
3	2.83	7.38	7.40	10.36
4	1.67	1.55	3.86	3.77
5	2.59	2.64	4.90	4.44
Aug. 6 and 8.				
1	1.92	2.04	2.54	2.40
2	4.45	8.71	6.02	11.04
3	3.42	8.07	6.78	11.25
4	1.11	1.10	2.76	2.68
5	1.67	7.46	2.43	8.67
Aug. 13 and 14				
1	1.68	1.82	1.98	1.95
2	4.43	8.14	7.11	10.05
3	3.51	7.63	7.33	10.54
4	.98	1.07	2.09	2.23
5	2.65	3.36	4.03	4.45
Aug. 23 and 24				
1	1.52	1.38	2.02	1.84
2	8.54	8.71	10.64	11.41
3	3.26	3.01	6.09	5.79
4	7.69	8.55	8.53	9.30
5	7.90	5.98	8.29	6.71

moisture was determined by drying to constant weight in an electric oven held at 105 ° C.

The official methods (*4*) were employed in obtaining the analytical results reported for starch, reducing sugars, sucrose, ash, phosphoric acid (P_2O_5), lime (CaO), magnesia (MgO), and sulfur (S). Corrections for sand and other siliceous soil material as applied in official methods (*4*) were used as a basis for calculating the results (see tables 9–13). Moisture in the leaf was determined by drying it at 100° C. for 4 hours. Nitrogen (N) was determined by the official Gunning method modified to include nitrate as given for fertilizers, with the substitution

of 0.7 gm. of mercuric oxide for copper oxide. Potash (K_2O) was determined by solution of sample by the wet digestion method of West (*19*) and the modified chloroplatinate method of Hicks (*11*). The weights of leaf material shown in tables 4 to 8 include the midrib and were not corrected for sand and other siliceous material, whereas the data shown in tables 9 and 10 were corrected for siliceous soil material and do not include the midrib. Nicotine was determined by a modification of the Keller method (*6*) made in the Division of Tobacco and Plant Nutrition.

EXPERIMENTAL RESULTS

PHYSICAL MEASUREMENTS

The water content of the surface soil was definitely modified by the methods used to control the water supply (table 2). It is inconceivable that the plants growing on the area to which no water was added and having a moisture content as low as 1 or 2 percent could have developed at all unless they were drawing on subsoil reserves. Soil-moisture content at greater depths shown in table 3 for 1937 indicated that this was taking place. The plants growing under these conditions as a rule exhibited severe wilting during the day and more or less wilting (fig. 1) at all times, but they did not show any considerable loss of the lower leaves from drying (see tables 4 and 5). At times during dry periods the water content of the soil of the control plot was as low as that of the soil of area 1, which received no rainfall and no irrigation. This condition was usually of short duration, however, since as a rule rain fell in time to correct this critical situation. When the water was withheld from area 3 late in the season, usually about 2 to 3 weeks before harvest, the soil moisture was rapidly reduced to a low level by the growing plants (tables 2 and 3).

TABLE 3.—*Soil-moisture content based on moist weight at depths in irrigation plots, Upper Marlboro, Md., 1937*

[Samples represent 12 borings per plot]

Date and treatment No.	Lowland (series A)		Upland (series B)		Date and treatment No.	Lowland (series A)		Upland (series B)	
	0–12 inches	12–24 inches	0–12 inches	12–24 inches		0–12 inches	12–24 inches	0–12 inches	12–24 inches
	Per-cent	*Per-cent*	*Per-cent*	*Per-cent*		*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*
July 28:					August 11—Con.				
1	6.75	13.73	3 33	5.31	4	3.50	9.61	3.43	4.81
2	8.60	11.42	7.77	7.85	5	5 38	8.16	4.09	3 86
3	7.82	11.99	7.97	7.80	August 18:				
4	5.62	12.05	3.96	5.54	1	3.16	10 00	2.35	3.76
5	6.36	9.76	3.81	5.54	2	8 47	11.45	7.49	3.92
August 4:					3	5.61	9.54	4.33	7.15
1	5.02	12.48	3 04	4.82	4	7.32	10.64	6.66	4.68
2	8.35	10.81	7.38	6.94	5	5.32	7.80	4.49	5.42
3	8.55	11.62	7.42	7.56	September 1:				
4	4.98	9.67	3 42	5.20	1	4.13	9.12	2.46	3.61
5	4.45	8.50	3.14	4.69	2	8.91	12.89	7.02	8.21
August 11:					3	6.55	8.29	3.86	4.92
1	3.69	11.40	2.61	4.05	4	8.10	15.22	7.08	8.78
2	7.95	10.55	6.22	7.10	5	8.28	12.27	6.28	7.32
3	7 53	10.67	6.51	6.67					

The actual growth rate of the plant can most satisfactorily be expressed in terms of measurements made at intervals of the height of the plant and of the width and length of the leaves and of counts of the leaves. Dry-weight data to determine at intervals the increase in dry matter necessitate the harvesting and destruction of part of the experimental plantings and so generally are not feasible.

The leaf area developed by the plants was determined at 14-day intervals. The method used was based upon the formula by Goff (*7, p. 374*):

> The area of a leaf was computed by multiplying its length by one-half its width, and increasing the product by one-third, it having been ascertained by trial that this is nearly exact. The average length and the average width of a leaf was ascertained by taking measurements of 800 whole leaves.

More recently a mathematical method for determining the area of tobacco leaves was reported by Gubenko (*8*). The methods discussed in his paper appear to agree very closely with Goff's formula $L \times 1/2\ W$ increased by 1/3, in which L represents the length and W the width of the leaf. This formula can also be expressed as $2/3\ L \times W$. While Goff used the average length and width in his calculations, possibly for leaves of nearly the same size, this procedure would not be accurate if the leaves varied greatly in size, as from the bottom to the top of the plant. The area of each leaf would then have to be determined as a unit and the total for the plant arrived at by summation; this procedure was followed to obtain the results reported in tables 4, 5, 7, and 8.

The areas of leaves by groups with reference to position on the stalk and the total leaf area per plant are shown for the two varieties of Maryland tobacco in tables 4 and 5. At the time of the first measurements, approximately 30 days after transplanting, comparatively little variation in size of plants on the several plots was shown by the data on total leaf area, height of plant, number of leaves, and length of internodes. The two varieties, Maryland Broadleaf and Maryland Medium Broadleaf, had leaf areas approximating 3 or 4 square feet per plant at the time of the first measurement, and the two varieties and the five treatments were in reasonably close agreement. The subsequent measurements, however, showed decided differences for both varieties as a result of the various water treatments. The use of supplemental water in treatment 2 almost doubled the leaf area per plant as compared with treatment 1 with neither rainfall nor irrigation. The Maryland Broadleaf consistently produced plants with a greater leaf area than the Maryland Medium Broadleaf. However, there was no decided difference in the area per plant in the dry and control treatments. There was a loss of area by the lower leaves as a result of drying, or firing. The leaf area finally attained in square feet per plant roughly paralleled the number of leaves from plants grown on areas which were supplied with water treatment 2 (throughout the period) or treatment 3 (only during early growth).

The plots to which additional water was supplied produced taller plants with longer internodes and more total leaves than those on the area from which water was withheld. The plants of the Maryland Broadleaf variety grown on areas kept dry during early growth had more green leaves than those on irrigated areas (table 4). The Maryland Broadleaf variety developed more leaves than the Maryland Medium Broadleaf and reached its maximum leaf area 2 weeks

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TABLE 4.—*Average area of green leaves, height of plant, length of internodes, and number of leaves per plant of Maryland Broadleaf tobacco grown with and without rainfall or supplemental water, Upper Marlboro, Md., 1936–38*

[Counts and measurements made at 14-day intervals on 24 plants comprising 2 rows, or one-third, of each plot in series B]

Measurement and treatment No.	Water treatment				Leaf area per plant								Height of plant	Average internode length	Leaves per plant		
	Precipitation		Irrigation		Groups of 5 leaves [1]							Total			Green	Dry	Total
	Early	Late	Early	Late	I	II	III	IV	V	VI	VII						
					Square feet	*Square feet*	*Square feet*	*Square feet*	*Square feet*	*Square feet*	*Square foot*	*Square feet*	*Inches*	*Inches*	*Number*	*Number*	*Number*
First:																	
1	Without	Without	Without	Without	1.18	1.59	0.29	0 02				2.99	5.94	0.53	11.21		11.21
2	With	With	With	With	1.28	1.57	22	01				3.08	5.95	.53	11.15		11.15
3	do	Without	do	Without	1.20	1.38	.18	.01				2 77	5 29	.50	10.54		10.54
4	Without	With	Without	With	1 27	1.17	.11					2.55	5.17	.51	10.05		10.05
5	With	do	do	Without	1.25	1.43	.30	.03				3.01	5 67	.52	10 91		10.91
Second:																	
1	Without	Without	do	do	1.22	3 49	3.10	.83	0.06			8.70	15 87	.92	16.30	1.03	17.33
2	With	With	With	With	1.41	4 94	5 57	2.27	.27	0.01		14.47	18.51	.90	19.19	1 28	20.47
3	do	Without	do	Without	1.41	4 72	4.92	1.71	.18			12.94	17.68	.92	18.22	.95	19.17
4	Without	With	Without	With	1 42	3 70	3 01	.57	.02			8.72	15.57	.93	16 30	.47	16.77
5	With	do	do	Without	1 32	4 38	4 60	1.84	.34	.01		12.49	17.38	92	17.49	1.37	18.86
Third:																	
1	Without	Without	do	do	88	3 69	4.61	2.70	93	.20		13.01	28.81	1 19	21.77	2.48	24.25
2	With	With	With	With	.61	5 14	8 04	7 12	4 05	1 44	0.14	26.54	45.34	1.51	26.22	3.85	30.07
3	do	Without	do	Without	.64	5.14	7 95	6 58	3 34	1.22	.13	25.00	43 24	1.41	26 98	3.71	30.69
4	Without	With	Without	With	1 12	4.04	4 99	3 00	1.09	.16		14.31	29.03	1.18	22.61	2.02	24.63
5	With	do	do	Without	.57	4.37	6.57	5 28	2.81	1.17	.17	20.94	39 13	1.42	23.72	3.87	27.59
Fourth:																	
1	Without	Without	do	do	.18	2 73	4.71	3.64	2.03	.20		13.49	30 93	1.22	20.31	5.06	25.37
2	With	With	With	With		2.27	7.04	8.14	6.11	1.60	.07	25.23	36.61	1.37	18.01	8.71	26.72
3	do	Without	do	Without	.03	2.37	7.29	7.87	5 83	1.17	.06	24.62	36.76	1.38	18.44	8.23	26.67
4	Without	With	Without	With	54	3.98	5.95	5.65	4 26	60	.02	21.00	33 72	1.29	22.13	4.02	26.15
5	With	do	do	Without	.01	2 30	5.75	6.56	4 54	.75	.04	19.95	32.92	1.26	18.07	8.15	26 22

[1] Bottom (I) and top (VII) groups did not always contain 5 leaves.

TABLE 5.—*Average area of green leaves, height of plant, length of internodes, and number of leaves per plant of Maryland Medium Broadleaf tobacco grown with and without rainfall or supplemental water, Upper Marlboro, Md., 1939–40*

[Counts and measurements made at 14-day intervals on 24 plants comprising 2 rows, or one-third, of each plot in series B]

Measurement and treatment No.	Water treatment				Leaf area per plant						Height of plant	Average internode length	Leaves per plant		
	Precipitation		Irrigation		Groups of 5 leaves [1]				Total	Harvested			Green	Dry	Total
	Early	Late	Early	Late	Bottom (lost)	Lower	Middle	Top							
					Square feet	*Square feet*	*Square feet*	*Square feet*	*Square feet*	*Square feet*	*Inches*	*Inches*	*Number*	*Number*	*Number*
First:															
1	Without	Without	Without	Without	1.86	1.76	0.13		3.75	1.89	5.67	3.50	11.27	0.17	11.44
2	With	With	With	With	3.54	.42	.02		3.98	.44	6.15	.53	11.37	.33	11.70
3	do	Without	do	Without	2.83	.88	.07		3.78	.95	5.53	.48	11.34	.21	11.55
4	Without	With	Without	With	1.97	1.32	.16		3.45	1.48	5.70	.50	10.99	.38	11.37
5	With	do	do	Without	2.27	.64	.04		3.95	.68	5.28	.46	11.40	.04	11.44
Second:															
1	Without	Without	do	do	1.93	4.57	2.47	0.26	9.23	7.30	13.13	.70	17.31	1.42	18.73
2	With	With	With	With	6.17	4.24	1.88	.30	12.59	6.42	14.99	.74	18.73	1.46	20.19
3	do	Without	do	Without	4.54	4.81	2.19	.49	12.33	7.79	14.25	.71	18.55	1.50	20.05
4	Without	With	Without	With	2.19	4.00	2.32	.36	8.87	6.68	12.64	.67	17.21	1.67	18.88
5	With	do	do	Without	4.37	4.28	1.90	.33	10.88	6.51	13.83	.72	17.84	1.40	19.24
Third:															
1	Without	Without	do	do	.95	4.23	3.06	.79	9.13	8.18	28.37	1.34	17.30	3.90	21.20
2	With	With	With	With	5.51	5.44	4.57	2.10	17.62	12.11	39.89	1.60	21.03	3.87	24.90
3	do	Without	do	Without	4.04	6.09	5.14	2.12	17.69	13.65	36.90	1.46	21.49	3.80	25.29
4	Without	With	Without	With	1.24	4.10	3.57	1.07	9.98	8.74	26.95	1.18	18.56	4.34	22.90
5	With	do	do	Without	3.40	5.18	3.55	1.22	13.35	9.95	33.65	1.43	19.54	4.02	23.56
Fourth:															
1	Without	Without	do	do	.26	4.11	4.46	2.96	11.79	11.53	20.11	.87	17.00	5.99	22.99
2	With	With	With	With	2.47	6.30	6.60	6.00	21.37	18.90	22.43	.92	17.22	7.26	24.48
3	do	Without	do	Without	2.14	6.34	7.13	6.10	21.71	19.57	23.06	.95	17.70	6.52	24.22
4	Without	With	Without	With	.42	4.11	4.69	4.86	14.08	13.66	19.41	.88	15.91	6.23	22.14
5	With	do	do	Without	1.43	5.79	6.40	5.38	19.00	17.37	20.51	.85	17.20	6.79	23.99

[1] Bottom and top groups did not always contain 5 leaves.

earlier, indicating more rapid growth (fig. 2). The Maryland Medium Broadleaf was apparently more seriously affected by the early dry period (treatment 4), since it did not recover as quickly or produce as much leaf area as the Maryland Broadleaf when irrigation was resumed late in the season (fig. 3). The total leaf number, internode

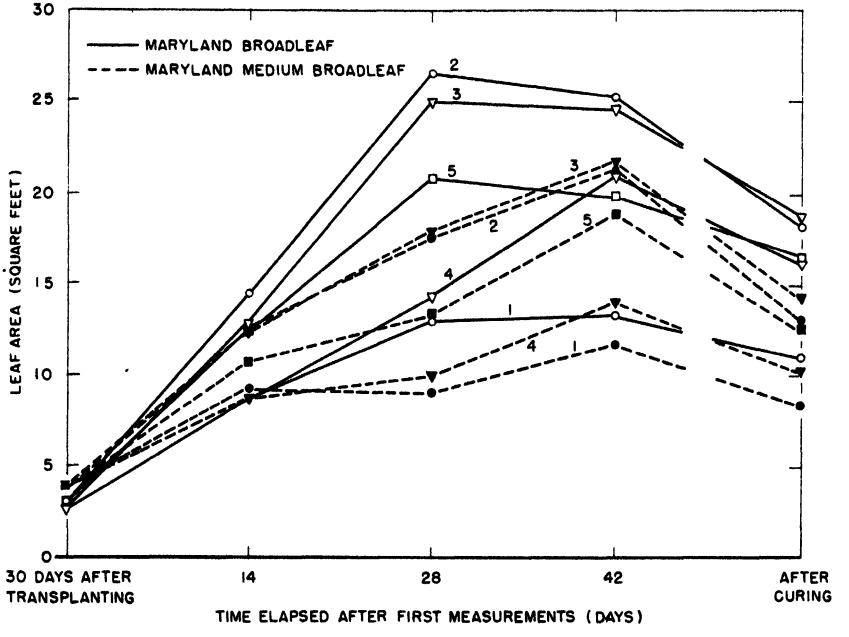

FIGURE 2.—Effect of irrigation on average leaf area per plant of the Maryland Broadleaf variety (1936–38) and the Maryland Medium Broadleaf (1939–40), when measured at 14-day intervals during the growing period and after curing. Data for cured leaf of the Maryland Medium Broadleaf are for 1939 only. Treatment 1, without rainfall or supplemental water during entire period of growth; treatment 2, with rainfall and supplemental water throughout period of growth; treatment 3, with rainfall and supplemental water during early period of growth, but without rainfall or supplemental water during late period of growth; treatment 4, without rainfall or supplemental water during early period of growth, but with rainfall and supplemental water during late period of growth; treatment 5, with rainfall, but without supplemental water during entire period of growth.

length, and height of plant showed much the same relation. The loss of the lower leaves was much more pronounced with both varieties on the plots furnished abundant moisture (rainfall and supplemental water).

A comparison of the leaves produced on dry soil and those produced on soil to which water was applied showed that the shape of the leaves was not greatly modified by the application of water. The ratio of length to width was practically constant except in the top leaves of Maryland Medium Broadleaf (table 6). The top leaves apparently increased in width where water was supplied. At the time of the fourth measurement the ratio of length to width was 2.41 with rainfall and supplemental water and 2.65 without either. The top leaves

were definitely longer in relation to width than the lower leaves. This relation changes gradually from the lower leaves, which were broad, to the middle leaves, which were narrow; the top leaves were the narrowest of the three groups. Although the addition of water did not greatly modify the shape of the leaf, it did cause a decided increase in size, as shown by measurements of length and width.

The measurements of the areas per plant of cured leaves, which possibly are not so accurate as those of the green-leaf areas, since it is difficult to smooth out cured leaves and obtain a true measure, showed decided differences as a result of the treatments (tables 7 and 8). The measurements were made and the data on moisture absorption were taken in a room where moisture and temperature

FIGURE 3.—Tobacco grown (*A*) without water during early growth, but with subsequent rainfall and supplemental water for 16 days and (*B*) with supplemental water and rainfall during early growth, but both subsequently withheld for 16 days. Photographed August 26, 1936. (Compare with fig. 1.)

were under control; they are, therefore, believed to be comparatively accurate. The moist and oven-dry weights of the cured leaves showed decided differences as a result of moisture supplied to the growing plants. The leaf of both varieties produced where water was withheld had the highest weight per square foot, but the Maryland Medium Broadleaf (table 8) had a higher weight per unit area than the Maryland Broadleaf (table 7). The 1940 crop was not included in the tabulation for cured leaf, as the control room was not available for handling the crop. As a rule the percentage of moisture absorbed by the cured leaf was highest where moisture was supplied in the field. Leaf grown on soil from which water was withheld early in the season was similar in moisture absorption and weight per square foot to that grown where water was withheld throughout the season (tables 7 and 8). Generally speaking, the leaf having the highest moisture-absorbing capacity and the lowest weight per square foot was produced on the plot from which water was withheld late in the season, that is, for 2 to 3 weeks before harvest (tables 7 and 8). The leaf produced on the control plot varied widely, depending upon the prevailing seasonal conditions.

TABLE 6.—*Average length and width of green leaves per plant of Maryland Medium Broadleaf tobacco grown with and without rainfall or supplemental water, Upper Marlboro, Md., 1939–40*

[Measurements made at 14-day intervals during growing season; usually 5 or 6 leaves per group of lower, middle, and top leaves per plant (average of 24 plants)]

Measurement and treatment No.	Water treatment				Lower leaves			Middle leaves			Top leaves		
	Precipitation		Irrigation		Length	Width	Ratio (length/width)	Length	Width	Ratio (length/width)	Length	Width	Ratio (length/width)
	Early	Late	Early	Late									
					Inches	*Inches*		*Inches*	*Inches*		*Inches*	*Inches*	
First:													
1	Without	Without	Without	Without	10.40	5.57	1.87	3.10	1.31	2.37			
2	With	With	With	With	7.28	3 34	2.18	2 28	.81	2.81			
3	do	Without	do	Without	8.16	4.10	1.99	3.36	1.38	2.43			
4	Without	With	Without	With	9.72	5.00	1 94	5 81	2.34	2.48			
5	With	do	do	Without	7.69	3.70	2.08	3.19	1.23	2.59			
Second:													
1	Without	Without	do	do	17.90	9.86	1.82	13.46	6.22	2.16	7.37	2.60	2.83
2	With	With	With	With	18.84	10.06	1.87	13.27	5.64	2.35	8.35	2 88	2.90
3	do	Without	do	Without	18.74	10 38	1.81	13 57	6 13	2.21	8 32	2.90	2.87
4	Without	With	Without	With	17.58	9.42	1.87	13.81	6.43	2 15	7 86	2.83	2.78
5	With	do	do	Without	18.18	9.88	1.84	12.94	5.78	2.24	8.67	2 96	2.93
Third:													
1	Without	Without	do	do	18 18	9.66	1.88	15.50	6.76	2 29	10.44	3.45	3.03
2	With	With	With	With	22.45	11.28	1.99	21.34	9.15	2.33	15.85	5.60	2.83
3	do	Without	do	Without	22.47	11 75	1.91	21.60	9.68	2.23	16.06	5.81	2.76
4	Without	With	Without	With	18.34	9 70	1.89	17.32	8.12	2.13	12.58	4.99	2.52
5	With	do	do	Without	20.96	10 63	1.97	18.03	7.61	2.37	12.53	4.33	2.89
Fourth:													
1	Without	Without	do	do	18 45	10 31	1.79	18.48	8.69	2.13	15.08	5.70	2.65
2	With	With	With	With	23 21	12.67	1.83	24.56	11.69	2.10	23.53	9.77	2 41
3	do	Without	do	Without	22 70	12 35	1.84	25.04	11.73	2.13	23.97	9.64	2.49
4	Without	With	Without	With	18.91	10.27	1.84	19 99	9.53	2 10	18 14	7.48	2.43
5	With	do	do	Without	21.42	11.93	1 80	23 37	11.26	2.08	21.90	8 98	2.44

The values shown in tables 4 and 7 are averages for 3 years and represent data for series B only. The values for individual years did not show a wide departure from the averages, and the relative response to the treatments was much the same each year. The values in table 5 are averages from series B for 2 years, and the values for individual years showed no apparent significant departures from the averages.

The stalk weights showed a greater difference between treatments than leaf weights; the stalks tended to be relatively smaller on the dry plot than on the irrigated ones, as shown by the percentage of top growth represented by the stalks (tables 7 and 8). Weights of stalks showed a similar trend (see table 14).

TABLE 7.—*Average area and moist and oven-dry weights of cured leaves per plant, moist and oven-dry weights per square foot of cured leaves, percentage of moisture absorbed by leaves when exposed to constant temperature and moisture conditions, and weights of stalks of Maryland Broadleaf tobacco grown with and without rainfall or supplemental water, Upper Marlboro, Md., 1936–38*

[Measurements made at 88 percent relative humidity and 77° F. on leaves from 24 plants comprising 2 rows, or one-third, of each plot in series B; the lower, middle, and top leaf groups usually consisted of 5 or 6 leaves per plant]

Plant part and treatment No.	Water treatment: Precipitation, Early	Water treatment: Precipitation, Late	Water treatment: Irrigation, Early	Water treatment: Irrigation, Late	Average area per plant	Moist weight	Oven-dry weight	Moisture	Moist weight per square foot	Oven-dry weight per square foot
					Square feet	*Grams*	*Grams*	*Percent*	*Grams*	*Grams*
Lower leaves:										
1	Without	Without	Without	Without	3 31	21 99	17 73	19 37	6 64	5 36
2	With	With	With	With	5 47	30 84	24 59	20 27	5 64	4 50
3	do	Without	do	Without	5 46	30 50	23 75	22 13	5 59	4 35
4	Without	With	Without	With	4 06	26 05	20 45	21. 50	6. 42	5 04
5	With	do	do	Without	4 64	27 36	21. 61	21. 02	5 90	4 66
Middle leaves:										
1	Without	Without	do	do	5 16	36 00	28 37	21 19	6 98	5 50
2	With	With	With	With	8. 16	52. 06	41 17	20 92	6 38	5 05
3	do	Without	do	Without	8 47	51. 24	39 57	22 78	6 05	4 67
4	Without	With	Without	With	7 13	43 45	33 56	22 76	6 09	4 71
5	With	do	do	Without	7 28	47. 05	36 64	22 13	6 46	5 03
Top leaves:										
1	Without	Without	do	do	2 62	22 93	17 56	23 42	8 75	6 70
2	With	With	With	With	4 56	35. 68	27 54	22 81	7 82	6 04
3	do	Without	do	Without	4 95	35 59	26 97	24 22	7 19	5 45
4	Without	With	Without	With	4. 88	36 63	27 37	25 28	7 51	5. 61
5	With	do	do	Without	4 27	32 52	24 74	23 92	7. 62	5. 79
All leaves:										
1	Without	Without	do	do	11 09	80. 92	63 66	21. 33	7 30	5. 74
2	With	With	With	With	18 19	118 58	93. 30	21 32	6. 52	5. 13
3	do	Without	do	Without	18 88	117 33	90 29	23 05	6. 21	4 78
4	Without	With	Without	With	16 07	106 13	81 38	23 32	6 60	5. 06
5	With	do	do	Without	16 19	106 93	82. 99	22 39	6. 60	5. 13
Stalks [1]					*Percent* [2]					
1	Without	Without	do	do	37 53	52. 77	38. 25	27 52		
2	With	With	With	With	48 76	115 08	88. 81	22 83		
3	do	Without	do	Without	42. 96	89 33	68. 01	23. 87		
4	Without	With	Without	With	40. 65	74. 92	55 73	25 61		
5	With	do	do	Without	46. 08	92. 87	70. 91	23. 65		

[1] Stalk weights are averages for 1937 and 1938.
[2] Percent of top (above-ground portion harvested).

The roots did not receive complete study, but among those studied there were decided differences in development (fig. 4). The roots of five plants from each treatment of the 1939 crop were washed out of the soil by a stream of water from a hose, and the average air-dry weights were determined (table 8). There were very few fibrous roots on plants grown on the area held dry all season (treatment 1), but abundant fibrous roots were evident where the plants were irrigated. Since it was not practical to wash out all the roots throughout their entire length, it was not possible to determine the effect of soil-water relations on root length.

TABLE 8.—*Average area and moist and oven-dry weights of cured leaves per plant, moist and oven-dry weights per square foot of cured leaves, percentage of moisture absorbed by leaves when exposed to constant temperature and moisture conditions, and weights of stalks and roots of Maryland Medium Broadleaf tobacco grown with and without rainfall or supplemental water, Upper Marlboro, Md., 1939*

Measurements made at 88 percent relative humidity and 77° F on leaves from 24 plants comprising 2 rows, or one-third, of plants for each treatment, the lower, middle, and top leaf groups usually consisted of 5 or 6 leaves per plant]

Plant part and treatment No.	Water treatment				Average area per plant	Moist weight	Oven-dry weight	Moisture	Moist weight per square foot	Oven-dry weight per square foot
	Precipitation		Irrigation							
	Early	Late	Early	Late						
					Square feet	*Grams*	*Grams*	*Percent*	*Grams*	*Grams*
Lower leaves:										
1	Without	Without	Without	Without	3.34	23.37	18.91	19.08	7.00	5.66
2	With	With	With	With	4.93	33.95	26.54	21.83	6.89	5.38
3	do	Without	do	Without	5.23	34.91	27.24	21.97	6.67	5.21
4	Without	With	Without	With	3.84	29.75	23.78	20.07	7.75	6.19
5	With	do	do	Without	4.35	32.21	25.19	21.80	7.40	5.79
Middle leaves:										
1	Without	Without	do	do	3.28	27.06	21.27	21.40	8.25	6.48
2	With	With	With	With	4.54	32.91	25.36	22.94	7.25	5.59
3	do	Without	do	Without	5.32	38.68	29.71	23.19	7.27	5.58
4	Without	With	Without	With	3.79	31.88	24.81	22.18	8.41	6.55
5	With	do	do	Without	4.66	35.41	27.13	23.38	7.60	5.82
Top leaves:										
1	Without	Without	do	do	1.82	16.60	12.89	22.35	9.12	7.08
2	With	With	With	With	3.72	29.96	22.81	23.87	8.05	6.13
3	do	Without	do	Without	3.82	30.34	23.07	23.96	7.94	6.04
4	Without	With	Without	With	2.73	22.84	17.55	23.16	8.37	6.43
5	With	do	do	Without	3.59	29.48	22.44	23.88	8.21	6.25
All leaves:										
1	Without	Without	do	do	8.44	67.03	53.07	20.83	7.94	6.29
2	With	With	With	With	13.19	96.82	74.71	22.84	7.34	5.66
3	do	Without	do	Without	14.37	103.93	80.02	23.01	7.23	5.57
4	Without	With	Without	With	10.36	84.47	66.14	21.70	8.15	6.38
5	With	do	do	Without	12.60	97.10	74.76	23.01	7.71	5.93
								Percent [1]		
Stalks:										
1	Without	Without	do	do	37.35			31.64		
2	With	With	With	With	43.64			57.84		
3	do	Without	do	Without	40.61			54.72		
4	Without	With	Without	With	31.96			31.07		
5	With	do	do	Without	41.88			53.87		
Roots:										
1	Without	Without	do	do		106.00	21.40	79.81		
2	With	With	With	With		314.00	63.90	79.65		
3	do	Without	do	Without		298.00	69.50	76.68		
4	Without	With	Without	With		181.00	33.60	81.44		
5	With	do	do	Without		193.00	37.40	80.62		

[1] Percent of top (above-ground portion harvested).

FIGURE 4.—Tobacco roots typical of those produced in irrigation series: *A*, Without rainfall or supplemental water during the entire period of growth; *B*, with rainfall and supplemental water throughout period of growth; *C*, with rainfall and supplemental water during early period of growth, but without either during late period of growth; *D*, without rainfall or supplemental water during early period of growth, but with both rainfall and supplemental water during late period of growth; *E*, with rainfall only during entire period of growth. Photographed October 1939.

BIOCHEMICAL STUDIES

The results presented in tables 9 and 10 are based on samples taken from series A (lowland) and B (upland) and represent average values corrected for the sand and other siliceous soil material adhering to the leaves. In most details the results from the two series agree as to effects produced by irrigation. The areas of leaves with midribs removed were determined by tracing on manila wrapping paper, measuring the total area of the paper, cutting out and weighing the leaf tracings, and calculating the areas on the basis of weight relations. The water supplied definitely increased the leaf area, green and oven-dry weights per leaf, water content, and green weight per square foot (table 9). These increases were as a rule directly related to the amount of water supplied by irrigation. The oven-dry weight per square foot in general was inversely related to the amount of water supplied. Much the same relations are apparent in table 10. The treatments for 1935 did not include a differential rate of irrigation, but did include differences in time of application. Withholding the water for 3 weeks prior to harvest (treatment 3, table 10) resulted in leaf weighing less per unit area than that from any other treatment represented, agreeing with the results presented in tables 7 and 8. However, in this instance the weight was much less since the results were corrected for sand and other siliceous soil material and the midrib weight was not included in the total area weight from which the calculations were made. It is interesting to note that the green weight per square foot of leaf area was lower in the area where water was withheld than in the area where water was added, supporting the values in the next column showing a greater weight per square foot of oven-dry material. This difference apparently was largely water, as the leaf showed a lower percentage of water. These differences became more pronounced as the season advanced; the August 23 samples show a wider difference than the July 23 samples.

764540—47——3

The results of analysis of the 1935 crop (table 11) are based on averages from series A and B, which were in reasonably close agreement. The percentage of nitrogen in the leaves, stalks, and tops of plants grown in areas from which water was withheld was definitely higher than in plants from areas supplied with water during early growth. However, the highest percentage of nitrogen was found in the leaves, stalks, and tops of plants grown on the area from which water was withheld early in the season and supplied during the last 3 weeks of growth (treatment 4). In fact, the percentages of all the ash constituents except sulfur were highest when the water was supplied only during the late period of growth. The percentage of phosphoric acid (P_2O_5), although low regardless of treatment, showed much the same relations as nitrogen. The percentage of potash (K_2O) was definitely lower in the leaves of plants grown in the area from which water was withheld than in areas where it was supplied, but the leaf grown under the late irrigations showed the highest content of this constituent. This increase in potash cannot be accounted for by the potash content of the water applied, as analysis of the water showed less than 4 pounds of K_2O applied per acre where supplemental water was supplied throughout the season. The data

TABLE 9.—*Average leaf area, green and oven-dry weights per leaf, water content, green and oven-dry weights per square foot of leaves from plants of Maryland Broadleaf tobacco grown with and without rainfall or supplemental water, Upper Marlboro, Md., 1934*

Samples consisted of 15 half leaves taken from 2 groups of 15 plants so as to avoid severe mutilation of plants values shown are averages from series A and B corrected for adhering sand and other siliceous soil material]

Date, leaf,[1] and treatment No	Water treatment		Average leaf			Water	Green weight per square foot	Oven-dry weight per square foot
	Precipitation	Irrigation	Area	Green weight	Oven-dry weight			
July 31 (seventh leaf):			*Square feet*	*Grams*	*Grams*	*Percent*	*Grams*	*Grams*
1	Without	Without	0.71	16 58	2.05	87.64	23 35	2.89
2	With	¼ inch twice weekly	76	19.66	2 43	87 74	25.87	3 20
3	do	½ inch twice weekly	.80	20 55	2.41	88 27	25 69	3.01
August 6 (seventh leaf):								
1	Without	Without	.78	17 59	2 55	85 50	22 55	3.27
2	With	¼ inch twice weekly	1.01	25.71	3 88	84 91	25 46	3 84
3	do	½ inch twice weekly	1.29	34.00	4.66	86 29	26.36	3.61
August 14 (eighth leaf):								
1	Without	Without	.88	20.32	2.67	86 86	23 09	3.03
2	With	¼ inch twice weekly	1 07	27 51	3.33	87 90	25 71	3 11
3	do	½ inch twice weekly	1.15	30.20	3.45	88.58	26 26	3.00
August 21 (eighth leaf):								
1	Without	Without	.87	18.66	2.64	85.85	21.45	3.03
2	With	¼ inch twice weekly	1.25	30.67	3.93	87.19	24.54	3.14
3	do	½ inch twice weekly	1.58	40.63	4.52	88.88	25.72	2.86
August 28 (ninth leaf):								
1	Without	Without	.97	22.29	3 16	85.82	22 98	3.26
2	With	¼ inch twice weekly	1 23	31.03	3.76	87.88	25.23	3.06
3	do	½ inch twice weekly	1.45	37.41	4.07	89.12	25.80	2.81
September 6 (ninth leaf):								
1	Without	Without	.89	18.32	2.94	83.95	20.58	3.30
2	With	¼ inch twice weekly	1.38	31.57	4.60	85.43	22.88	3.33
3	do	½ inch twice weekly	1.72	42.87	4.91	88.55	24.92	2.85

[1] Leaves numbered from bottom of plant.

thus indicate an important influence of water upon the availability to the tobacco plant of relatively insoluble soil potash. The percentages of lime and magnesia were increased by withholding water, but the reverse appears to be true of sulfur. However, when water was added late in the season the percentages of lime and magnesia were increased. The plants from the control treatment tended to approach those that received the late additions of water in percentage content of the various constituents. The percentage of total ash content was lowest in the leaf of plants grown where water was supplied throughout the growing period and highest where water was supplied during the last 3 weeks prior to harvest. These same relations prevailed for the stalks and consequently for the tops, including leaf and stalk.

The actual recovery values for ash constituents (pounds per acre) were related much the same as the percentage values were

TABLE 10.—*Average leaf area, green and oven-dry weights per leaf, water content, green and oven-dry weights per square foot of leaves from plants of Maryland Broadleaf tobacco grown with and without rainfall or supplemental water, Upper Marlboro, Md., 1935*

Samples consisted of 15 half leaves taken from 2 groups of 15 plants so as to avoid severe mutilation of plants; values shown are averages for series A and B corrected for adhering sand and other siliceous soil material]

Date, leaf,[1] and treatment No.	Water treatment				Average leaf			Water	Green weight per square foot	Oven-dry weight per square foot
	Precipitation		Irrigation		Area	Green weight	Oven-dry weight			
	Early	Late	Early	Late						
					Square feet	*Grams*	*Grams*	*Per-cent*	*Grams*	*Grams*
July 23 (seventh leaf).										
1	Without	Without	Without	Without	0.49	10.73	1.82	83 04	21 90	3.71
2	With	With	With	With	.67	15.76	2 25	85 72	23. 52	3 36
3	do	Without	do	Without	.73	16.71	2.41	85 58	22 89	3.30
4	Without	With	Without	With	.54	11.71	1.94	83.43	21.69	3 59
5	With	do	do	Without	.77	18. 71	2. 72	85. 46	24 30	3. 53
July 30 (seventh leaf):										
1	Without	Without	do	do	.59	14. 08	2. 68	80 97	23. 86	4. 54
2	With	With	With	With	.88	21. 83	3 46	84. 15	24. 81	3. 93
3	do	Without	do	Without	.93	23 33	3 69	84. 18	25. 09	3 97
4	Without	With	Without	With	.59	13. 44	2. 49	81. 47	22. 78	4 22
5	With	do	do	Without	.96	24. 80	3. 87	84. 40	25. 83	4. 03
August 6 (eighth leaf):										
1	Without	Without	do	do	.62	14. 16	2. 82	80. 08	22. 84	4. 55
2	With	With	With	With	1 13	28. 90	4. 39	84 81	25. 58	3. 88
3	do	Without	do	Without	1. 05	26. 43	4 12	84. 41	25 17	3. 92
4	Without	With	Without	With	.63	14. 15	2 70	80 92	22 46	4. 29
5	With	do	do	Without	1. 09	26 88	4. 10	84. 75	24. 66	3. 76
August 13 (eighth leaf):										
1	Without	Without	do	do	.66	15 22	2 93	80. 75	23. 06	4. 44
2	With	With	With	With	1. 08	26. 50	3. 97	85. 02	24. 54	3. 68
3	do	Without	do	Without	1. 06	24. 89	3 74	84. 97	23. 48	3. 53
4	Without	With	Without	With	.58	13 56	2. 62	80. 68	23. 38	4. 52
5	With	do	do	Without	1. 05	25. 49	3 90	84. 70	24. 28	3. 71
August 23 (ninth leaf):										
1	Without	Without	do	do	.70	15. 92	3 20	79 90	22. 74	4. 57
2	With	With	With	With	1. 28	31 34	4. 62	85. 26	24. 48	3. 61
3	do	Without	do	Without	1. 14	26. 66	3. 84	85. 60	23 39	3. 37
4	Without	With	Without	With	.85	21. 29	3. 34	84. 31	25. 05	3 93
5	With	do	do	Without	1. 24	31. 37	4. 70	85. 02	25. 30	3. 79

[1] Leaves numbered from bottom of plant.

TABLE 11.—*Average weight per plant, content and recovery per acre of the different plant-food constituents, and content and yield of nicotine from Maryland Broadleaf tobacco grown with and without rainfall or supplemental water, Upper Marlboro, Md., 1935*

[Determinations based upon 15 sample plants from each of series A and B; values are averages on a moisture-free basis and corrected for sand and other siliceous soil material]

Plant part and treatment No	Water treatment				Average weight per plant	Content							
	Precipitation		Irrigation			Total ash	Nitrogen (N)	Phosphoric acid (P_2O_5)	Potash (K_2O)	Lime (CaO)	Magnesia (MgO)	Sulfur (S)	Nicotine
	Early	Late	Early	Late									
Leaves:					*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	Without	Without	Without	Without	31.70	14.85	2.57	0.62	4.40	3.29	0.75	0 69	1.36
2	With	With	With	With	56.04	14.75	2.16	.51	5.07	3.17	.64	1.00	.75
3	do	Without	do	Without	43.84	16 08	2 10	.53	5.55	3.10	.56	.92	.76
4	Without	With	Without	With	50.94	18.83	3.25	.67	5.67	4.27	.84	.88	.99
5	With	do	do	Without	59.78	16.01	2.54	.58	5.33	3 32	.71	.93	1.05
Stalks:													
1	Without	Without	do	do	17.83	8.63	2 67	.67	3.89	1.09	.41	.31	.37
2	With	With	With	With	53.89	7.88	2.32	.65	3.60	.94	.34	.39	.25
3	do	Without	do	Without	40.50	8.68	2.53	.70	3.90	1.04	.34	.44	.30
4	Without	With	Without	With	30.49	12.07	3.45	.81	5.75	1.37	44	.40	.37
5	With	do	do	Without	50.93	8.52	2.79	.66	3 89	.97	.38	.38	.31
Tops (leaves and stalks):													
1	Without	Without	do	do	49.54	12.64	2.60	.64	4.21	2.51	.62	.56	1.02
2	With	With	With	With	109 93	11.38	2 24	.57	4.35	2.08	.50	.70	.52
3	do	Without	do	Without	84.34	12.54	2.31	.61	4.76	2.11	.46	.69	.54
4	Without	With	Without	With	81.43	16.30	3.33	.72	5.71	3 31	.69	.70	.75
5	With	do	do	Without	110.71	12 57	2.66	.61	4.67	2.24	.56	.67	.72

Plant part and treatment No.	Water treatment				Recovery per acre							Nicotine yield per acre
	Precipitation		Irrigation		Total ash	Nitrogen (N)	Phosphoric acid (P_2O_5)	Potash (K_2O)	Lime (CaO)	Magnesia (MgO)	Sulfur (S)	
	Early	Late	Early	Late								
					Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
Leaves:												
1	Without	Without	Without	Without	56.47	9 72	2 34	16 79	12.48	2.80	2.57	5.11
2	With	With	With	With	99.18	14 47	3 35	34.00	21 41	4.29	6.67	5.15
3	do	Without	do	Without	84.78	11 10	2.70	29.21	16.45	2 94	4.83	3 97
4	Without	With	Without	With	114 88	19.93	4 06	34.67	26.05	5.04	5.38	6 00
5	With	do	do	Without	114.44	18.18	4.10	38 07	23.76	5.03	6.58	7. 54
Stalks:												
1	Without	Without	do	do	18.28	5.71	1.42	8.34	2.24	.82	.62	.80
2	With	With	With	With	50 96	14.97	4 10	23 25	6.17	2.17	2.54	1.58
3	do	Without	do	Without	42 73	12.50	3 36	19.17	5.17	1.69	2.17	1.49
4	Without	With	Without	With	43.98	12 55	2.95	20.96	4 97	1.61	1.43	1.34
5	With	do	do	Without	51.98	16.96	4.00	23.74	5.86	2.31	2.25	1.88
Tops (leaves and stalks).												
1	Without	Without	do	do	74 75	15.43	3 76	25 13	14.72	3.62	3.19	5.91
2	With	With	With	With	150 14	29.44	7 45	57.25	27.58	6.46	9.21	6.73
3	do	Without	do	Without	127.49	23 60	6 06	48.38	21 62	4.63	7.00	5.46
4	Without	With	Without	With	158.86	32.48	7 01	55 63	31.02	6.65	6.81	7.34
5	With	do	do	Without	166.42	35 14	8 10	61.81	29 62	7.34	8.83	9.42

Plant part and treatment No.	Water treatment				Area	Weight per square foot								
	Precipitation		Irrigation			Dry matter	Total ash	Nitrogen (N)	Phosphoric acid (P_2O_5)	Potash (K_2O)	Lime (CaO)	Magnesia (MgO)	Sulfur (S)	Nicotine
	Early	Late	Early	Late										
Leaves.					*Square feet*	*Grams*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*
1	Without	Without	Without	Without	5 55	5 819	0. 863	0 149	0. 036	0 255	0. 191	0 043	0. 040	0. 079
2	With	With	With	With	11 04	5 072	750	109	.025	257	.162	.032	.051	.039
3	do	Without	do	Without	9. 45	4 653	747	.098	.024	.258	.143	.026	.043	.036
4	Without	With	Without	With	10 96	4 645	.875	151	.031	263	.198	.039	.041	.046
5	With	do	do	Without	11 15	5 364	.858	.137	.031	.285	.179	.038	.049	.057

(table 11). However, there was one notable exception: the greatest recovery of total ash and most constituents that make up the ash took place in plants on the control plot. The lowest took place consistently where the soil was held dry during the entire period (treatment 1). However, on the basis of recovery per square foot of leaf surface, the leaves grown under the dry condition early in the season showed higher recovery of all constituents except potash and sulfur than those grown under other treatments.

The results of the analysis of the 1936 crop from series B (table 12) showed much the same relations as those for 1935 (table 11). The crop grown under dry conditions (treatment 1) had the highest percentage of nitrogen and the greatest content per unit area of leaf, but a lower recovery in pounds per acre than the crop that received treatment 5 because of the small size of the plants. The percentage of potash was about the same in the leaf produced on the control plot as in that grown under irrigation, but the highest total recovery in pounds per acre took place where supplemental water was added. Again, the lowest recovery in pounds per acre took place in plants grown where water was withheld. The main difference appears to be one of size of plant rather than increased percentage of any constituent. The exceptions to this generalization were the highest percentage of nitrogen in leaf of plants grown for the entire period with low soil water (treatment 1) and the lowest percentage of nitrogen in the leaf grown under irrigation (treatment 2), apparently the result of leaching from the soil.

In both 1935 and 1936 the percentages of nicotine in the leaf and stalk of plants grown without water (treatment 1) were distinctly higher than in those of plants grown with irrigation throughout the season. Nicotine content per unit area of leaf was also highest in plants grown without water. The application of water late in the season produced a higher percentage of nicotine as well as a larger amount per square foot of leaf area than other irrigation treatments. The percentage of nicotine was lowest where there was continuous irrigation. These comparisons seem to bear some relation to the nitrogen content. The yields of nicotine on the acre basis did not show the same relation to the treatments that the percentages did because of compensation in size of plants.

Some studies were made to determine the effect of irrigation on the organic constituents of tobacco (table 13). In the late stages of growth starch and sucrose were definitely higher in the leaf grown under dry conditions (treatment 1) than in that grown with abundant moisture (treatment 2). Leaf composition of control plants was similar to that of leaf subjected to treatment 1. There was apparently a difference in the content of reducing sugars.

YIELDS, VALUE, AND QUALITY OF THE CROP

The yields and value of the crop are in the last analysis the best tangible measure of the product. The crop must be acceptable to the trade to become an economic factor in agriculture. It is not always possible or easy to obtain a true picture of yield and value relations, but carefully conducted tests for a period of years offer the best available approach. The results presented in table 14 show striking

TABLE 12.—*Average weight per plant, content and recovery per acre of the different plant-food constituents, and content and yield of nicotine from Maryland Broadleaf tobacco grown with and without rainfall or supplemental water, Upper Marlboro, Md., 1936*

[Determinations based upon 24 sample plants from series B (moisture-free basis and corrected for sand and other siliceous soil material)]

Plant part and treatment No.	Water treatment				Average weight per plant	Content							
	Precipitation		Irrigation			Total ash	Nitrogen (N)	Phosphoric acid (P_2O_5)	Potash (K_2O)	Lime (CaO)	Magnesia (MgO)	Sulfur (S)	Nicotine
	Early	Late	Early	Late									
					Grams	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Leaves:													
1	Without	Without	Without	Without	40 93	17.01	3 93	0 57	5 25	4 02	1.01	0 54	2.10
2	With	With	With	With	79.79	18 46	2.02	53	6 76	3.76	.80	70	1 39
5	do	do	Without	Without	69 90	17 84	2.81	.57	7 04	3 09	.94	.63	1.92
Stalks:													
1	Without	Without	do	do	23 58	10 09	2.99	.54	4 85	1 18	.39	.23	.44
2	With	With	With	With	65 62	9 79	2 49	.69	4.86	.97	.35	.31	.39
5	do	do	Without	Without	50 99	10.10	3.08	.69	5 20	.85	.40	29	.47
Tops (leaves and stalks):													
1	Without	Without	do	do	64. 51	14 48	3. 59	.56	5 10	2 98	.78	.43	1 49
2	With	With	With	With	145 41	14 55	2 23	.60	5 90	2 50	.60	.53	.93
5	do	do	Without	Without	120. 89	14 58	2 92	.62	6 26	2 15	.71	.48	1 31

Plant part and treatment No.	Water treatment				Recovery per acre							
	Precipitation		Irrigation		Total ash	Nitrogen (N)	Phosphoric acid (P_2O_5)	Potash (K_2O)	Lime (CaO)	Magnesia (MgO)	Sulfur (S)	Nicotine yield per acre
	Early	Late	Early	Late								
					Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
Leaves:												
1	Without	Without	Without	Without	133 24	19 28	2 80	25 70	19 66	4.94	2 64	10 27
2	With	With	With	With	281 92	19. 24	5 01	64 47	35 92	7.67	6 75	13. 20
5	do	do	Without	Without	228 75	23 44	4 72	58 88	25 90	7 81	5 27	16. 08
Stalks												
1	Without	Without	do	do	45 54	8. 44	1 51	13 69	3 34	1. 11	.66	1. 23
2	With	With	With	With	122 96	19 56	5 47	38. 10	7 60	2. 77	2. 46	3. 01
5	do	do	Without	Without	94 46	18 83	4 21	31. 71	5 14	2. 42	1. 73	2. 85
Tops (leaves and stalks)												
1	Without	Without	do	do	178 78	27. 72	4 31	39 39	23 00	6 05	3. 30	11 50
2	With	With	With	With	404 88	38 80	10 48	102 57	43 52	10 44	9 21	16. 21
5	do	do	Without	Without	323 21	42. 27	8 93	90 59	31 04	10 23	7. 00	18. 93

TABLE 12.—*Average weight per plant, content and recovery per acre of the different plant-food constituents, and content and yield of nicotine from Maryland Broadleaf tobacco grown with and without rainfall or supplemental water, Upper Marlboro, Md., 1936*—Continued

Plant part and treatment No.	Water treatment				Weight per square foot								
	Precipitation		Irrigation		Dry matter	Total ash	Nitrogen (N)	Phosphoric acid (P_2O_5)	Potash (K_2O)	Lime (CaO)	Magnesia (MgO)	Sulfur (S)	Nicotine
	Early	Late	Early	Late									
Leaves:					*Grams*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*
1	Without	Without	Without	Without	5.278	0.897	0.208	0.030	0.277	0.212	0.053	0.028	0.111
2	With	With	With	With	5.077	.937	.102	.027	.343	.191	.041	.036	.070
5	do	do	Without	Without	4.890	.872	.137	.028	.344	.151	.046	.031	.094

TABLE 13.—*Average area, green weight, dry weight, moisture content, dry matter per square foot, and percentage of starch, reducing sugars, and sucrose of leaves of Maryland Medium Broadleaf tobacco grown with and without rainfall or supplemental water, Upper Marlboro, Md., 1939*

Date and treatment No.	Water treatment				Average leaf area	Average green weight [1]	Average dry weight [1]	Total moisture [1]	Dry matter per square foot [1]	Green basis [1]		
	Precipitation		Irrigation							Starch	Reducing sugars	Sucrose
	Early	Late	Early	Late								
					Square feet	*Grams*	*Grams*	*Percent*	*Grams*	*Percent*	*Percent*	*Percent*
Aug. 13:												
1A	Without	Without	Without	Without	0 80	17 94	2 66	85. 18	3 33	0 97	0. 47	0. 25
2A	With	With	With	With	97	24 39	3. 54	85. 49	3 64	1 11	40	.24
5A	do	do	Without	Without	.91	21 68	3. 33	84 64	3. 68	.76	.67	.29
Aug. 28: [2]												
1A	Without	Without	do	do	81	18. 03	2 69	85. 09	3 33	1 05	.26	.28
2A	With	With	With	With	1 11	26 61	3 55	86. 64	3. 20	1 68	.55	.26
5A	do	do	Without	Without	1 05	26 31	3 90	85. 18	3. 73	1. 58	.41	.25
Sept. 14:												
1A	Without	Without	do	do	.71	16 31	2 83	82 65	4. 01	2 50	.45	.36
2A	With	With	With	With	1 07	23 39	3. 38	85 55	3. 17	1 63	.51	.21
5A	do	do	Without	Without	1 07	25 94	4 09	84. 24	3 81	2 02	.46	.34

Date and treatment No.	Water treatment				Moisture-free basis [1]			Weight per square foot of leaf area		
	Precipitation		Irrigation		Starch	Reducing sugars	Sucrose	Starch	Reducing sugars	Sucrose
	Early	Late	Early	Late						
					Percent	*Percent*	*Percent*	*Gram*	*Gram*	*Gram*
Aug. 13:										
1A	Without	Without	Without	Without	6 57	3 16	1 71	0 219	0. 105	0 057
2A	With	With	With	With	7 66	2 78	1 63	279	.101	.059
5A	do	do	Without	Without	4 97	4 35	1 90	183	.160	.070
Aug. 28: [2]										
1A	Without	Without	do	do	7 03	1 73	1 91	.234	.058	.064
2A	With	With	With	With	12 55	4 08	1 94	.401	.131	.062
5A	do	do	Without	Without	10 67	2 73	1 71	.398	.102	.064
Sept. 14.										
1A	Without	Without	do	do	14 41	2 62	2 08	.578	.105	.083
2A	With	With	With	With	11 27	3 50	1 45	.357	111	.046
5A	do	do	Without	Without	12 80	2 94	2 18	.488	.112	.083

[1] Corrected for sand and other siliceous soil material.
[2] Time of flowering and topping.

TABLE 14.—*Yields of leaf and stalk, gross values, and prices of tobacco grown with and without rainfall or supplemental water, Upper Marlboro, Md., 1935–40*

Series and treatment No.	Water treatment				Yield of leaf tobacco per acre						
	Precipitation		Irrigation		1935	1936	1937	1938	1939	1940	Average
	Early	Late	Early	Late							
Series A:					*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
1	Without	Without	Without	Without	788	782	876	650	400	438	656
2	With	With	With	With	738	1,076	936	745	810	813	853
3	do	Without	do	Without	682	851	960	738	800	751	797
4	Without	With	Without	With	926	1,013	876	750	675	751	832
5	With	do	do	Without	919	1,213	840	688	790	688	856
Series B:											
1	Without	Without	do	do	462	744	860	750	602	663	680
2	With	With	With	With	888	1,226	1,140	963	1,036	1,014	1,045
3	do	Without	do	Without	894	1,188	1,300	1,038	1,218	1,038	1,113
4	Without	With	Without	With	751	1,100	1,300	975	574	851	925
5	With	do	do	Without	869	1,125	1,200	1,000	1,036	913	1,024
Both series:											
1	Without	Without	do	do	625	763	868	700	501	551	668
2	With	With	With	With	813	1,151	1,038	854	923	914	949
3	do	Without	do	Without	788	1,020	1,130	888	1,009	895	955
4	Without	With	Without	With	839	1,057	1,088	863	625	801	872
5	With	do	do	Without	894	1,169	1,020	844	913	801	940

Series and treatment No	Water treatment				Yield of tobacco stalks per acre						
	Precipitation		Irrigation		1935	1936	1937	1938	1939	1940	Average
	Early	Late	Early	Late							
Series A:					*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
1	Without	Without	Without	Without	381	375	616	400	240	238	375
2	With	With	With	With	569	763	960	638	660	688	713
3	do	Without	do	Without	431	538	768	531	560	575	567
4	Without	With	Without	With	488	538	752	475	370	450	512
5	With	do	do	Without	694	725	816	598	610	513	658

Series B:											
1	Without	Without	do	do	188	375	470	675	280	363	392
2	With	With	With	With	681	875	1,060	1,050	854	775	883
3	do	Without	do	Without	575	813	960	1,025	903	813	848
4	Without	With	Without	With	269	613	1,040	738	252	500	569
5	With	do	do	Without	569	650	910	700	728	625	697
Both series.											
1	Without	Without	do	do	285	375	543	538	260	301	384
2	With	With	With	With	625	819	1,010	844	757	732	798
3	do	Without	do	Without	503	676	864	778	732	694	708
4	Without	With	Without	With	379	576	896	607	311	475	541
5	With	do	do	Without	632	688	863	644	669	569	678

Series and treatment No.	Water treatment				Average proportion of leaf (1935–40)	Value of leaf tobacco per acre							Average price per pound (1935–40)
	Precipitation		Irrigation			1935	1936	1937	1938	1939	1940	Average	
	Early	Late	Early	Late									
Series A:					*Percent*	*Dollars*	*Dollars*	*Dollars*	*Dollars*	*Dollars*	*Dollars*	*Dollars*	*Cents*
1	Without	Without	Without	Without	64	63	152	159	131	68	98	112	17.07
2	With	With	With	With	54	179	310	185	154	243	356	238	27.90
3	do	Without	do	Without	58	153	229	178	146	216	321	207	25.97
4	Without	With	Without	With	62	124	221	121	137	116	217	156	18.75
5	With	do	do	Without	57	227	362	179	128	191	273	227	26.52
Series B:													
1	Without	Without	do	do	63	25	141	76	167	83	188	113	16.62
2	With	With	With	With	54	225	423	277	240	288	428	314	30.05
3	do	Without	do	Without	57	270	410	184	251	295	465	313	28.12
4	Without	With	Without	With	62	90	293	134	210	40	171	156	16.86
5	With	do	do	Without	60	201	320	223	272	237	340	266	25.98
Both series													
1	Without	Without	do	do	64	44	147	118	149	76	143	113	16.92
2	With	With	With	With	54	202	367	231	197	266	392	276	29.08
3	do	Without	do	Without	57	212	320	181	199	256	393	260	27.23
4	Without	With	Without	With	62	107	257	128	174	78	194	156	17.75
5	With	do	do	Without	58	214	341	201	200	214	307	246	26.17

and consistent differences in yield and gross value per acre of tobacco. These differences were the result of variation in rainfall and additions of supplemental water to the field-grown crop. There necessarily are variations in the results from year to year and between the two soils, but the yields and values obtained show somewhat the same relations each year.

Possibly the most notable variations were shown by tobacco grown on the control plot under normal rainfall (fig. 5, *A*); sometimes the yield was as large and the value as great as for crops produced with additional water. However, this is to be expected, since under ideal distribution of rainfall the results from the control treatment could even exceed those obtained from irrigation, as the excess water might cause

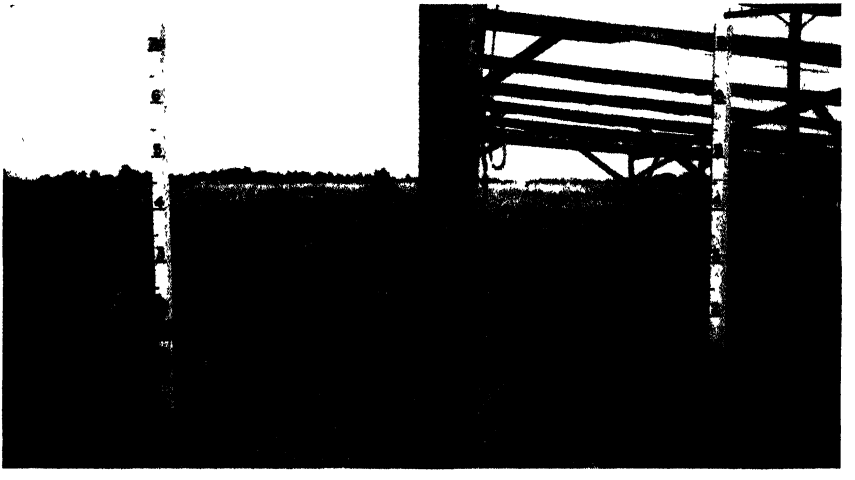

FIGURE 5.—Tobacco grown (*A*) under prevailing rainfall and (*B*) without rainfall or supplementary water. Border plants were removed just prior to harvest. The scaffolding shown was for the support of the canvas. Photographed August 26, 1936.

leaching and reduce the yields and values; if the season were unusually dry, however, the results might approach those from area 1, which received no water from rainfall or irrigation. The highest average value was obtained by the addition of supplemental water as required throughout the growing season (fig. 6, *A*); the highest yield and the next highest value were obtained for tobacco from the plot where water was withheld late in the season. The control plot gave the next highest average yield and value. This was followed by those of the plot which received water only late in the season. The lowest yields and values were obtained from area 1 from which water was withheld during the entire growing period. These same relations are evident in the average price per pound; the highest price obtained was for the leaf from the irrigated plot.

The stalk yields showed much the same relations as the leaf yields, but the highest percentage of leaf was found with the lowest yields (treatments 1 and 4) and the lowest percentage of leaf was found with the highest yields (treatments 2 and 3). Although a large stalk is not

desirable, it appears that with the present varieties the stalk yield increased out of proportion to the leaf yields.

The average value of the crop for the years covered by the test indicates that there is no great advantage in using supplemental water in addition to normal rainfall. It should be pointed out that the rainfall was usually adequate during the years covered by the test; however, if such a season as 1930 or 1943 had occurred, the results from irrigation would have been more striking. It seems to be clear that a higher quality product is consistently produced, as indicated by the average price per pound, by the use of irrigation as a supplement to natural rainfall. If economic conditions should change so that high-quality leaf would demand a greater premium, the use of

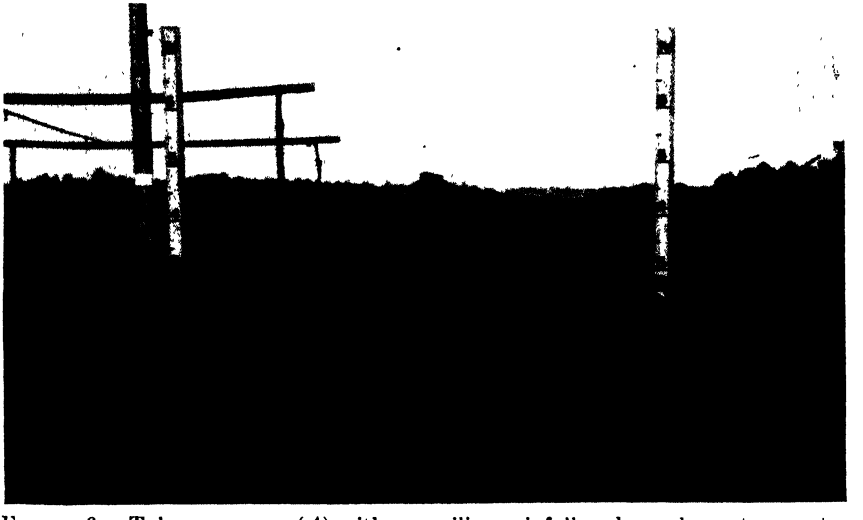

FIGURE 6.—Tobacco grown (*A*) with prevailing rainfall and supplementary water when required and (*B*) with the prevailing rainfall as the only source of water. Photographed August 26, 1936.

irrigation might be more profitable. These relations are further brought out in table 15, in which percentage of leaf for each grade is shown. These grades do not represent equal samples, but they indicate quality relationships. The high percentage of dull, or tip, grade indicates immaturity of leaf (treatments 1 and 4).

It is generally recognized by the trade that good fire-holding capacity is a prime requisite of Maryland tobacco. The high quality of the leaf produced as a result of irrigation is well illustrated by table 16, in which the fire-holding capacity is shown. The leaf produced on the plot kept dry during the entire period showed a low fire-holding capacity, as did the leaf grown on the plot that got late irrigation. There was apparently some reduction in fire-holding capacity of the leaf produced on the area kept dry for only 2 to 3 weeks before harvest. Where supplemental water was added throughout the period of growth, the leaf showed the highest fire-holding capacity. As was to be expected, the leaf grown on the control area

TABLE 15.—*Proportion of leaf tobacco in each farm grade in crops grown with and without rainfall and supplemental water, Upper Marlboro, Md., 1935–40*

Series and treatment No.	Water treatment				Bright (middle leaves)						
	Precipitation		Irrigation								
	Early	Late	Early	Late	1935	1936	1937	1938	1939	1940	Average[1]
					Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Series A:											
1	Without	Without	Without	Without	53	37	41	40	40	54	44
2	With	With	With	With	54	50	50	52	62	66	55
3	do	Without	do	Without	53	49	35	47	50	63	49
4	Without	With	Without	With	51	47	41	33	44	62	47
5	With	do	do	Without	56	56	43	45	58	58	53
Series B:											
1	Without	Without	do	do	45	39	40	47	40	62	45
2	With	With	With	With	59	66	58	52	55	63	59
3	do	Without	do	Without	58	61	52	53	49	67	57
4	Without	With	Without	With	50	52	34	44	35	46	43
5	With	do	do	Without	53	51	55	55	50	68	55
Both series:[1]											
1	Without	Without	do	do	50	38	40	43	40	59	44
2	With	With	With	With	57	59	54	52	58	64	58
3	do	Without	do	Without	56	56	45	51	50	66	53
4	Without	With	Without	With	51	50	37	39	40	53	45
5	With	do	do	Without	55	54	50	51	54	64	54

Series and treatment No.	Water treatment				Seconds (lower leaves)						
	Precipitation		Irrigation								
	Early	Late	Early	Late	1935	1936	1937	1938	1939	1940	Average[1]
					Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Series A:											
1	Without	Without	Without	Without	21	20	14	16	22	23	19
2	With	With	With	With	23	23	15	16	24	22	21
3	do	Without	do	Without	24	22	15	17	23	22	20
4	Without	With	Without	With	15	18	14	20	23	20	18
5	With	do	do	Without	17	18	21	21	22	26	20

Series B:											
1	Without	Without	do	do	23	20	16	30	16	19	21
2	With	With	With	With	19	14	16	19	19	14	17
3	do	Without	do	Without	20	13	14	17	20	16	16
4	Without	With	Without	With	18	16	14	20	20	13	17
5	With	do	do	Without	18	16	15	18	16	18	17
Both series: [1]											
1	Without	Without	do	do	22	20	15	24	19	21	20
2	With	With	With	With	21	18	16	18	21	17	18
3	do	Without	do	Without	21	17	14	17	21	18	18
4	Without	With	Without	With	16	17	14	20	22	16	17
5	With	do	do	Without	17	17	18	19	19	21	18

Series and treatment No.	Water treatment				Dull (top leaves)						
	Precipitation		Irrigation								
	Early	Late	Early	Late	1935	1936	1937	1938	1939	1940	Average [1]
Series A:					*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	Without	Without	Without	Without	26	43	45	44	38	23	37
2	With	With	With	With	23	27	35	32	14	12	24
3	do	Without	do	Without	23	29	50	36	27	15	31
4	Without	With	Without	With	34	35	45	47	33	18	35
5	With	do	do	Without	27	26	36	34	20	16	27
Series B:											
1	Without	Without	do	do	32	41	44	23	44	19	34
2	With	With	With	With	22	20	26	29	26	23	24
3	do	Without	do	Without	22	26	31	30	31	17	27
4	Without	With	Without	With	32	32	52	36	45	41	40
5	With	do	do	Without	29	33	30	27	34	14	28
Both series: [1]											
1	Without	Without	do	do	28	42	45	33	41	20	36
2	With	With	With	With	22	23	30	30	21	19	24
3	do	Without	do	Without	23	27	41	32	29	16	29
4	Without	With	Without	With	33	33	49	41	38	31	38
5	With	do	do	Without	28	29	32	30	27	15	28

[1] Calculated from original data.

was erratic in fire-holding capacity from season to season, but on the average it approached that of the leaf grown on the area irrigated throughout the season.

TABLE 16.—*Fire-holding capacity of leaf tobacco grown with and without rainfall and supplemental water, Upper Marlboro, Md., 1934–36 and 1938–39*

Series and treatment No.	Water treatment				Average duration of glow					
	Precipitation		Irrigation		1934	1935	1936	1938	1939	Average [1]
	Early	Late	Early	Late						
					Seconds	*Seconds*	*Seconds*	*Seconds*	*Seconds*	*Seconds*
Series A:										
1	Without	Without	Without	Without	3.8	4.7	9.3	3.5	6.0	5.9
2	With	With	With	With	13.3	26.1	37.4	19.7	11.0	23.6
3	do	Without	do	Without	33.5	13.1	28.8	12.1	7.1	15.3
4	Without	With	Without	With	.....	5.1	8.0	3.3	5.6	5.5
5	With	do	do	Without	.....	12.5	34.4	24.6	10.6	20.5
Series B:										
1	Without	Without	do	do	6.9	4.8	6.8	24.3	5.6	10.4
2	With	With	With	With	10.7	12.6	25.6	67.5	15.7	30.4
3	do	Without	do	Without	48.1	28.4	26.8	55.5	9.8	30.1
4	Without	With	Without	With	.....	10.1	6.9	37.6	7.2	15.5
5	With	do	do	Without	.....	7.4	16.6	87.4	5.9	29.3
Both series:										
1	Without	Without	do	do	5.4	4.8	8.1	13.9	5.8	8.2
2	With	With	With	With	12.0	19.4	31.5	43.6	13.4	27.0
3	do	Without	do	Without	40.8	20.8	27.8	33.8	8.5	22.7
4	Without	With	Without	With	.....	7.6	7.5	20.5	6.4	10.5
5	With	do	do	Without	.....	10.0	25.5	56.0	8.3	24.9

[1] 1934 results not included.

DISCUSSION

The studies to determine the effects of irrigation were carried out on typical tobacco soils of southern Maryland; the heavier soils of this section were not represented. In other words, these tests were conducted on light soils that are subject to the leaching common under field conditions. Heavy soils are generally poorly suited to the production of Maryland tobacco and many other types because waterlogging sometimes occurs. Irrigation, or the use of supplemental water, on heavy soils if followed by heavy rainfall might result in insufficient oxygen for proper root development; it might even cause destruction of the greater part of the functioning roots. The overhead irrigation system used in this experiment was preferred since it washes the leaves much as rainfall does and is the desirable method for use on light soils subject to leaching.

There is reason to believe that the leaf tobacco produced on the areas held dry for the entire period of growth was not the typical dry-season product, since it was grown under very extreme conditions. In dry seasons soil is usually wet for a time and then is extremely dry for a period longer than the 2 to 3 weeks prior to harvest that certain areas in these experiments were kept dry. It is also true that atmospheric humidity above the dry soil area was not reduced, whereas it would be reduced during a dry season. However, the addition of water late in the season to an area which had been held dry for 2 to 3 weeks before harvest resulted in the succulent immature or second growth which produced cured leaf of poor quality.

Anderson and his associates (*1, 2, 3*) recognized the effect of leaching where irrigation is practiced and used nitrates to offset it. It should be recognized that accurate control of supplies of nitrate as well as of all other nutrient relations is desirable, but such control is difficult if not impossible to attain under field conditions. It is chiefly these relations that produce responses in any field study of irrigation effects on growth of tobacco.

The effects of irrigation during the early part of the season or throughout it on weight per square foot of leaf area are consistent in that leaf produced on irrigated plots weighed less than that produced on plots from which water was withheld. This would appear to indicate a lower density per unit area, as the leaves were definitely larger. The striking and consistent effect of irrigation in increasing the potash content of the leaf may be a partial explanation of the high quality of the product. Previous work (*15*) had shown a product of higher quality where liberal potash applications were made.

The higher nicotine content of leaf produced under dry conditions would appear to contradict the results previously reported when tobacco was grown for studies of nicotine production (*14*). The earlier tests demonstrated that irrigation generally increased the nicotine content, other factors being equal. However, it should be pointed out that the tests under discussion were conducted on soils of low fertility, particularly low in nitrogen, whereas the previous tests were conducted on highly fertile soil.

The improvement in fire-holding capacity of the leaf associated with abundant moisture from rainfall and supplemental water throughout the growing period appears to be an established fact. Such leaves had a lower weight per unit area as well as a higher content of potash.

SUMMARY

Irrigation experiments with tobacco were carried out on liberally fertilized loamy sand and sandy loam, typical tobacco soils of southern Maryland. Precipitation was withheld by means of a movable canvas from one area and supplementary water was added when required to another area in an attempt to simulate dry- and wet-weather conditions simultaneously on adjacent areas. The treatments were continuous throughout the season on two areas and were reversed late in the season on two others. The responses in growth and composition of tobacco subjected to the four treatments were compared with those of tobacco subjected to the prevailing weather conditions on a fifth area.

A larger leaf with a lower weight per unit area was produced when irrigation was used as a supplement to rainfall during the early part of the season or throughout it. The leaf area per plant produced with irrigation was about 25 square feet for the Maryland Broadleaf variety and 21 square feet for the Maryland Medium Broadleaf variety; these values were approximately double those for leaves produced where water was withheld during most of the growing period.

The cured leaf from tobacco subjected to irrigation treatments absorbed a higher percentage of moisture when exposed in a constant-temperature and humidity room than did leaf grown under dry conditions.

The most outstanding differences in composition of leaf were the higher potash percentage in leaf from plants grown on irrigated areas and the higher nitrogen percentage in the leaf from plants grown under dry conditions throughout the growth period or during early growth. The leaf produced under dry conditions during early growth or throughout the growing period was also definitely higher in nicotine; the nicotine content paralleled the higher nitrogen content. The highest percentage of ash was found in the leaf produced on areas to which water was supplied during the last 2 to 3 weeks before harvest and the lowest ash in leaf produced on areas from which water was withheld for most of the growing period.

The average yield, value, and price per pound of leaf tobacco were consistently in favor of using supplemental water with rainfall rather than withholding both. The advantage from the use of supplemental water, when compared with rainfall only, did not show up so decisively or so consistently, since during the period covered by this test the rainfall was generally almost adequate for normal growth. Nevertheless, on the average, there was a small increase in value when supplemental water was used during the early part of the growing period and throughout it. Withholding water during the early part or throughout the growing period resulted in a leaf of poor quality associated with immaturity, as indicated by the high percentage of leaf in the dull, or tip, grade.

The fire-holding capacity of the leaf was strikingly and consistently improved by the use of supplemental water during early growth in addition to rainfall.

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EFFECT OF INITIAL ACIDITY ON CALCIUM AND MAGNESIUM REQUIREMENTS OF TOBACCO IN ASEPTIC CULTURE [1]

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INTRODUCTION

A great deal of investigational work has been done on the relation of acidity to the growth of plants. A summary by Russell [2] mentioned the fact that a slight degree of acidity is usually beneficial in solution culture. He continued with the statement: "In soils, on the other hand, plants make their best growth in neutral or nearly neutral conditions." Definite evidence is available, however, that this generalization is too broad and that acidity may or may not be beneficial in either growth medium.[3] A summary by Pettinger [4] seems to indicate that many soils suitable for good crop production are acid in character. These writers emphasized not acidity in itself, but its influence on availability of nutrients. Similar evidence was presented by Albrecht and Schroeder.[5] Arnon and Johnson [6] found in addition that an increase in calcium ions could compensate for decreased availability of calcium due to excessive acidity. Acidity of the growth medium, therefore, would seem to be only one of many factors influencing availability, and not the all-important factor it was first considered.

A brief study, therefore, has been made on the relation of acidity in the range pH 4 to 7 to the calcium and magnesium requirements of seedlings of Xanthi Turkish tobacco (*Nicotiana tabacum* L.) in aseptic culture under controlled environmental conditions. The absence of extraneous micro-organisms in such studies is not usually considered important, although no evidence for this assumption is known. The data obtained with increasing quantities of calcium and magnesium at several initial acidities of the nutrient solution are presented in the form of growth curves.

[1] Received for publication January 27, 1947.

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EXPERIMENTAL PROCEDURE

Xanthi Turkish tobacco seedlings were grown on 50 cc. of a mineral-salt solution in 200-cc. Pyrex Erlenmeyer flasks under aseptic conditions. The temperature used was 25° C., and light of about 500 foot-candles was furnished by 3,500° white fluorescent lamps. The growth period was 28 days.

The mineral-salt solution consisted of water, 1,000 cc.; $Ca(NO_3)_2.4H_2O$, 1.444 gm.; $Mg.(NO_3)_2.6H_2O$, 0.318 gm.; K_2HPO_4, 0.366 gm.; $KHSO_4$, 0.085 gm.; and NH_4Cl, 0.072 gm. Separate stock solutions of calcium nitrate, magnesium nitrate, and potassium phosphate plus potassium bisulfate plus ammonium chloride in 20× concentration were used in the preparation of the mineral-salt solution. In preparing cultures with varying quantities of calcium ion, calcium nitrate was replaced with 1.039 gm. of sodium nitrate ($NaNO_3$) and calcium was added as the chloride. Magnesium nitrate, similarly, was replaced with 0.211 gm. of sodium nitrate in the study of magnesium concentrations. The base solution, therefore, contained nitrogen, 225 mg.; potassium, 189 mg.; phosphorus, 65 mg.; magnesium, 30 mg.; calcium, 245 mg.; and sulfur, 20 mg. per liter. Micronutrients, except for boron (H_3BO_3), were added to this solution as the chlorides. The quantities used were iron, 15 mg.; zinc, 0.5 mg.; copper, 0.125 mg.; manganese, 1.0 mg.; and boron, 0.5 mg. per liter. Acidity was adjusted with 0.1 N hydrochloric acid.

The composition of the mineral-salt solution was equivalent to that used by McMurtrey [7] in solution-culture studies with tobacco. It differed only in that potassium was increased from 125 to 189 mg. per liter and in that potassium nitrate, monopotassium phosphate, and magnesium sulfate were replaced with dipotassium phosphate and potassium bisulfate, the other salts being readjusted in concentration.

The cultures in the magnesium series contained 57.1 mg. of sodium ion per liter and those in the calcium series 281.1 mg. in addition to all the essential elements. Addition of hydrochloric acid for pH adjustment totaled not more than 28 mg. of chloride ion per liter, as compared with the 31 mg. originally present as ammonium chloride. Use of the chlorides of magnesium and calcium further increased the chloride-ion content by a maximum of 29.2 and 442.3 mg. per liter, respectively, depending on the particular concentration of magnesium or calcium ion employed.

The tobacco seeds were germinated in sterilized petri dishes containing a layer of blotting paper and several layers of filter paper. Seeds sterilized by immersion in 1:1,000 silver nitrate solution for 15 minutes were washed several times with sterile distilled water and then poured as a water suspension into the petri dishes. With a flamed platinum needle the seedlings were transferred to sterile Erlenmeyer flasks containing the nutrient medium and deposited on a double layer of filter paper held in a plant holder. This holder consisted of a glass rod 4 mm. in diameter which passed through the absorbent cotton in the neck of the flask and had a glass ring fused to its lower end. The filter-paper disks were held in this ring by means of a loose inner glass ring as shown in figure 1. The edges of the paper

[7] McMURTREY, J. E., Jr. DISTINCTIVE EFFECTS OF THE DEFICIENCY OF CERTAIN ESSENTIAL ELEMENTS ON THE GROWTH OF TOBACCO PLANTS IN SOLUTION CULTURES. U. S. Dept. Agr. Tech. Bul. 340, 43 pp., illus. 1933.

disks were bent down and forced between the rings to keep them in position and were then perforated. The purpose of the holder was

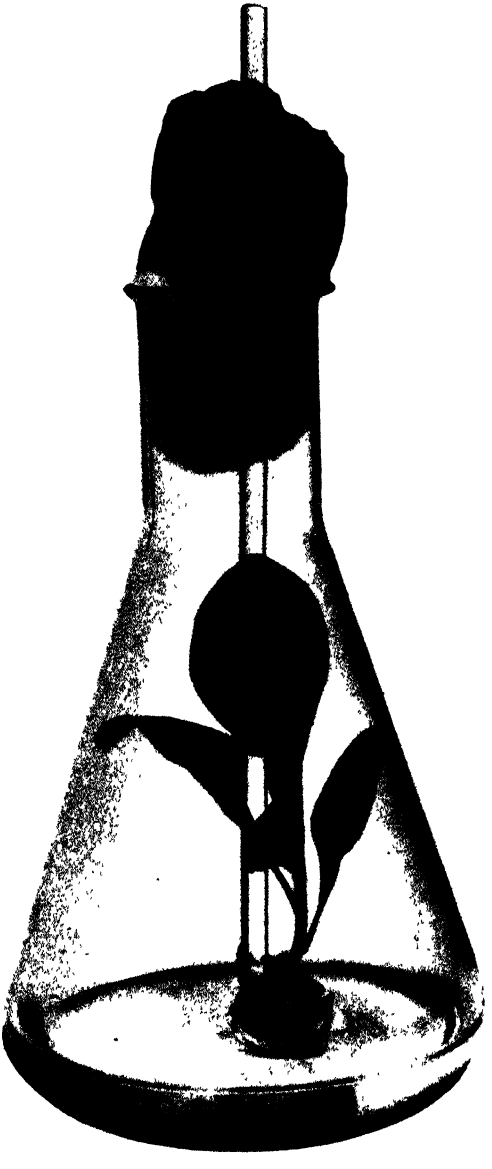

FIGURE 1.—Three-week-old seedling of Xanthi Turkish tobacco growing under aseptic conditions. A glass rod with fused-on ring passes through the absorbent-cotton plug. Two filter-paper disks, which are perforated with a needle after insertion in holder, are held in place in the ring by means of a loose inner glass ring, the edges of the paper disks being forced between the two rings.

to prevent contact of all but the roots of the plant with the solution. All glassware and media were sterilized at 15 pounds' pressure for 30 minutes.

At harvest the seedlings were washed, dried in the oven at 103° to 105° C. for 4 hours, cooled in the desiccator, and then weighed.

Two of the four duplicate seedlings were weighed together as a unit in each determination. Statistical methods appeared inapplicable, since it was necessary to reject about 10 percent of the seedlings because of contaminations with micro-organisms and unintentional injuries during transfer to the flasks. Some injured seedlings did not grow out of the cotyledon stage.

INFLUENCE OF ACIDITY ON CALCIUM REQUIREMENT

The effects obtained by varying the calcium content on the growth of the seedlings at four levels of acidity are shown in figure 2. The initial acidity levels were pH 6.48, 5.98, 5.42, and 4.39. Each value for dry weight is the average for eight seedlings, or for four in each of two determinations. The acidities at harvest were obtained by mixing the four residual solutions in each run and averaging the pH values in both runs. The averages found in this manner are not true pH values, but the deviations for the small variations encountered are probably well within those of experimental error.

It will be observed that in the solution at pH 6.48 the calcium optimum for growth was about 100 mg. per liter and that residual acidity of the solution increased with calcium content. The increase in acidity persisted even with decreasing yields. At an acidity level of pH 5.98 the growth curve was much flatter because of the slightly increased yields at deficiency levels of 25 and 50 mg. of calcium per liter. A contributing factor was a moderate decrease in maximum yield. Acidities at harvest also increased at this initial pH, but less than at pH 6.48.

INFLUENCE OF ACIDITY ON MAGNESIUM REQUIREMENT

The relation of magnesium requirements to acidity of the nutrient solution is shown in figure 3. Determinations were made at three levels of initial acidity—pH 5.96, 5.31, and 4.34. The number of repetitions and the method of averaging values were the same as those in the calcium series. At pH 5.96 the maximum yield was obtained with 6 mg. of magnesium per liter. Increasing acidity decreased maximum yields only slightly and did not alter the optimum magnesium concentration. Increasing acidities also caused slight decreases in yield with suboptimum concentrations of magnesium. At harvest acidities were in all cases approximately the same and were influenced but little by the initial acidity of the nutrient solution.

DISCUSSION

Adjustments in hydrogen-ion concentration and magnesium or calcium content of the nutrient solution are of course not feasible without alterations in other constituents. Moreover, it seemed advisable in these experiments under aseptic conditions to follow the usual procedure of using sodium and chloride ions, that is, sodium nitrate, hydrochloric acid, and the chlorides of magnesium and calcium. The basis for this procedure is the apparent nonessentiality of sodium and chlorine for growth of green plants. Nevertheless, sodium and chloride ions cannot be assumed a priori to be without influence on growth. Similar series employing fluctuations in essential ions might also be

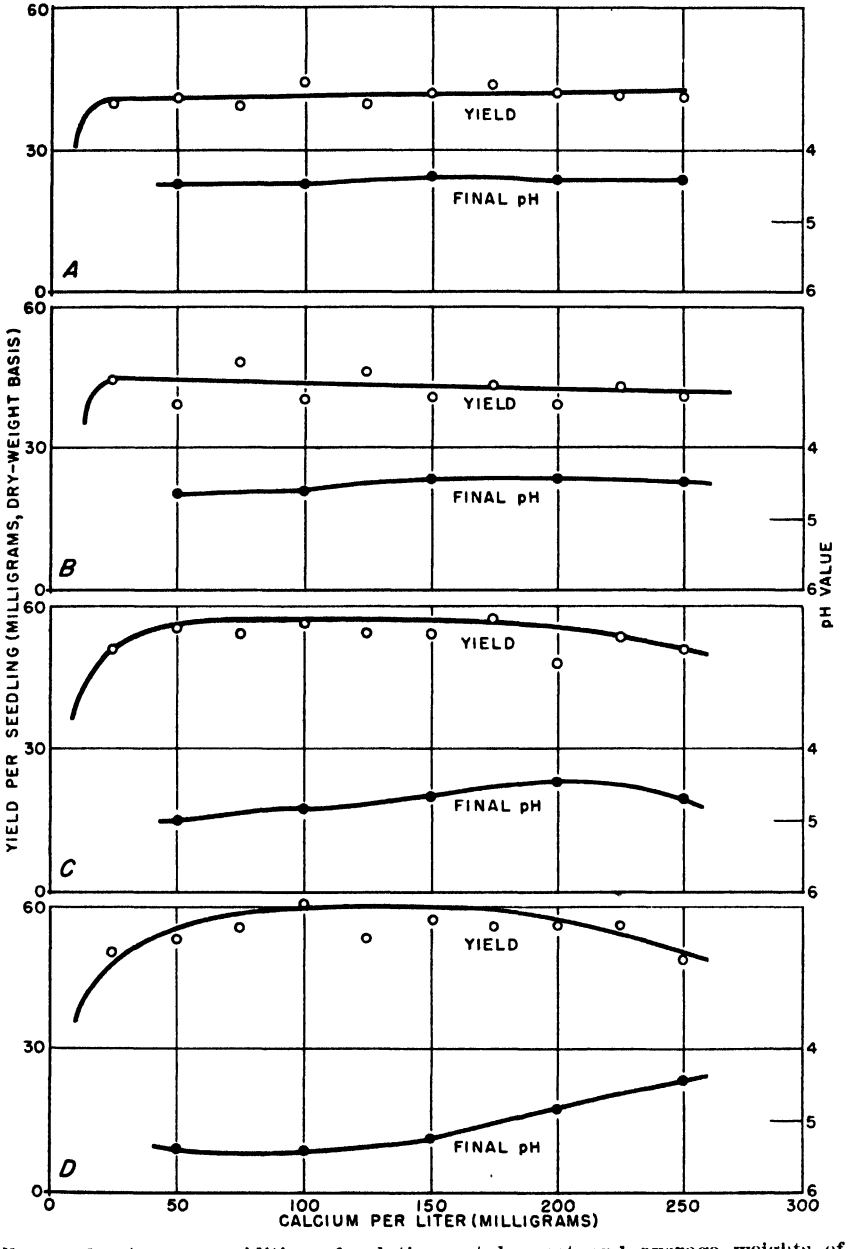

FIGURE 2.—Average acidities of solutions at harvest and average weights of Xanthi Turkish tobacco seedlings grown for 28 days with continuous illumination of 500 foot-candles in nutrient solutions containing different amounts of calcium and having different initial acidity levels: *A*, pH 4.39; *B*, pH 5.42; *C*, pH 5.98; and *D*, pH 6.48.

desirable for comparison, though it would be necessary to use initial excesses in order to avoid deficiencies.

The degree of influence of sodium and chloride ions in the calcium and magnesium series is indicated in several ways by the experimental

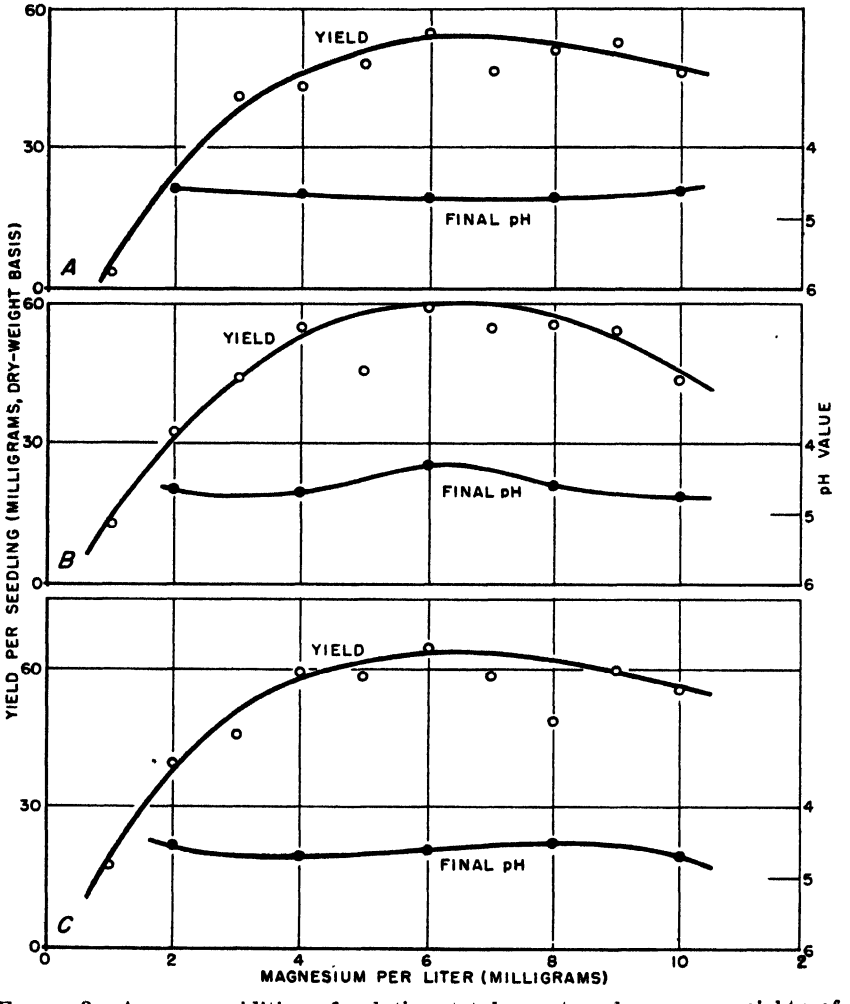

FIGURE 3.—Average acidities of solutions at harvest and average weights of Xanthi Turkish tobacco seedlings grown for 28 days with continuous illumination of 500 foot-candles in nutrient solutions containing different amounts of magnesium and having different initial acidity levels: *A*, pH 4.34; *B*, pH 5.31; *C*, pH 5.96.

data. The maximum yields in the two series were 63 mg. for magnesium and 60 mg. for calcium at the highest initial pH employed. The effects of a fivefold increase in sodium ion and of a sixfold increase in chloride ion as between the series are therefore rather small. Moreover, though the highest concentrations of chloride are concomitant

with those of magnesium and calcium, the depressions in yield at high nutrient levels were greatest in the magnesium series containing only one-fifth the chloride content. Furthermore, no symptoms of injury attributable to sodium or chlorine could be detected in either series.

The effects of increasing acidities on requirements of Xanthi Turkish tobacco seedlings were not entirely uniform for calcium and magnesium. With magnesium maximum yield decreased with increasing acidities within an initial range of pH 5.96 to 4.34. Since the optimum concentration of magnesium for growth remained unaltered, the magnesium requirement was thereby increased slightly. Increasing acidity also decreased maximum yield with calcium concentrations and so also increased the calcium requirement for growth. Increased acidity, however, increased yields at suboptimum concentrations of calcium and thus brought about a *relative* decrease in calcium requirements at intermediate acidity levels in the more acid series. At an initial acidity of pH 6.48, for example, yields with 25, 50, 75, and 100 mg. of calcium per liter were 49.6, 52.8, 56.4, and 61.2 mg., respectively; whereas the corresponding yields for an initial acidity of pH 5.98 were 51.3, 54.4, 54.5, and 56.3 mg. There was also a slight increase in absolute values for yields in the more acid series at suboptimum levels as compared with the less acid series. Furthermore, it should be noted that maximum yield was attained with 75 mg. of calcium with an initial pH of 5.98, whereas 100 mg. of calcium was required at pH 6.48.

These data cannot, however, be considered as proof that growth responses to calcium and magnesium display an intrinsic qualitative difference. The ranges used were not identical; that for magnesium extended from 0 to 166.67 percent of the optimum, whereas that for calcium was 0 to 250 percent of the optimum. Moreover, it is not certain but that the concentrations of other macronutrients and of the micronutrients used in the nutrient solution form the basis for these qualitative differences.

These data seem to indicate that acidity is not necessarily beneficial in a solution culture but that its action is dependent on the composition of the nutrient solution. Maximum yields were obtainable without resorting to an increased acidity to increase availability of nutrient ions. On the other hand, if a stock solution of much lower calcium content had been used, it is evident that increased acidity would have proved beneficial in the calcium series and perhaps also in the magnesium series. These statements might be summarized by stating that acidity may prove harmful to growth if all nutrient ions are present in ample quantity, but beneficial if there is a deficiency of calcium and perhaps of other elements.

SUMMARY

Xanthi Turkish tobacco seedlings were grown aseptically on 50 cc. of a mineral-salt solution in 200-cc. Erlenmeyer flasks at 25° C. with 500 foot-candles of white fluorescent illumination. The calcium and magnesium optima for growth were determined at several levels of initial acidity within the range pH 4 to 7 (adjusted with hydrochloric acid). Increased acidity brought about increased calcium and magnesium (as chlorides) requirements by decreasing growth with identi-

cal supply of these elements. Growth decreases were greater with increased acidity in the calcium series than in the magnesium series. Moreover, although the concentration-yield curves for magnesium remained practically unaltered in form with varying acidity, the analogous calcium curves tended to become straight. That is, the optimum for magnesium remained unaltered whereas that for calcium decreased with acidity. The residual solutions at harvest were usually slightly more acid than the unused nutrient solutions and were rather uniform in acidity under varying conditions.

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POPULATION DISTRIBUTION OF THE BEET LEAFHOPPER IN RELATION TO EXPERIMENTAL FIELD-PLOT LAY-OUT [1]

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INTRODUCTION

In field experiments to determine the relative merits of different insecticides or other treatments for controlling insect infestations, experimental designs such as the randomized block and the Latin square are frequently used. Such designs reduce the error of the experiment by restricting each comparison of unlike treatments to a limited part of the experimental field, thus giving treatment comparisons that are in a measure independent of location differences in the degree of infestation.

These designs are also well adapted to analysis of variance (*10*).[3] By this method of analysis the error reduction achieved by the design of the experiment may be evaluated and removed from the estimate of experimental error, thus increasing the precision with which the effects of treatment may be measured. Restricted designs are therefore justified on the general assumption that their use, together with the proper method of analysis, will significantly reduce the error variance, and thereby increase the efficiency of the experiment.

The nature of the distribution of the beet leafhopper (*Eutettix tenellus* (Baker)) in a particular field of sugar beets was studied on different dates, and with the data obtained from sampling beet-field populations, the relation between experimental design and the distribution of insect populations was determined.

MATERIALS AND METHODS

In May 1937 a field approximately 4½ acres in area near Grand Junction, Colo., planted with U. S. No. 34 sugar beets (*Beta vulgaris* L.) resistant to curly top was selected for the experiment. The field was divided into 36 equal-sized plots to form a 6×6 Latin square (fig. 1).

Each plot was 91 feet long and 60 feet (36 rows) wide, the rows being spaced 20 inches apart. The area of a plot was thus about ⅛

[1] Received for publication April 28, 1947.

[2] The author is indebted to W. C. Cook for suggestions and encouragement in the preparation of this manuscript, and to Myron E. Hall, who assisted in obtaining the field samples

[3] Italic numbers in parentheses refer to Literature Cited, p. 277.

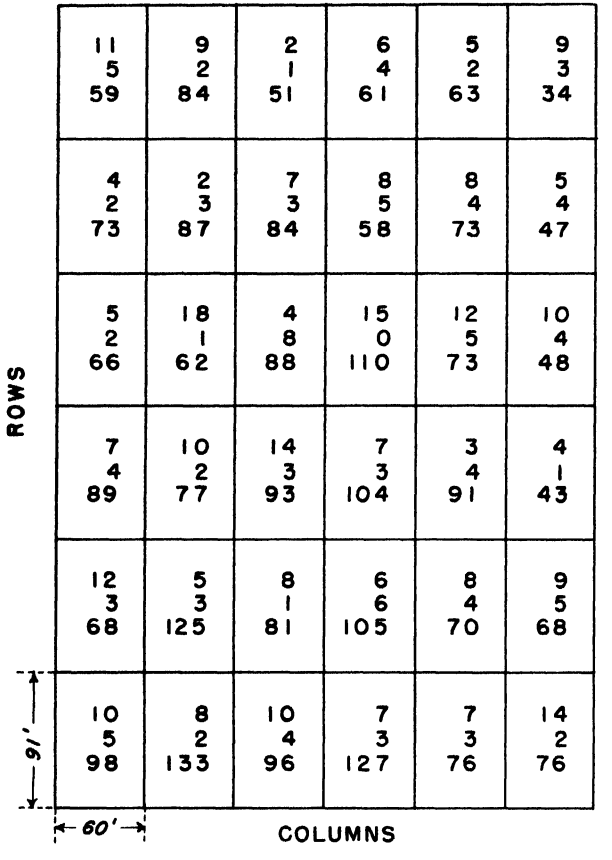

FIGURE 1.—Diagram of experimental field giving the total number of adult beet leafhoppers found on 10 beets in each plot on May 20 (upper figure); June 4 (middle figure); and August 26–27 (lower figure).

acre. At the time of thinning, the beets were spaced 10 inches apart along the rows.

Quantitative samples of the beet leafhopper population were obtained with a square-foot sampling cage essentially the same as that described by Hills (*14*). The first series of samples was taken in the field on May 20, 1937, shortly after the second influx of migrant beet leafhoppers into the Grand Valley fields, and about the time that the infestation was at its peak. The field was sampled again on June 4, when the migrant populations were on the decline, and again on August 26–27, after there had been brood development within the field. Ten square-foot cage samples were taken at random[4] in each plot on each date, or a total of 360 samples for the field. The adult leafhoppers were counted and recorded.

[4] Samples were chosen by moving about the plot with the head and eyes down, stopping at irregular intervals, and at each stop selecting the leafhoppers from the sixth beet along the row from the beet nearest the toe of the right foot.

The data obtained at each sampling were fitted to various theoretical distributions and tested by χ^2.

A study of the plot variation and the effect of local control in reducing experimental error was made by the method of analysis of variance. All of these analyses are on a single-sample basis.

NATURE OF THE DISTRIBUTION OF BEET LEAFHOPPER POPULATIONS

EARLY SEASON MIGRANT POPULATION

The nature of the sampling data suggests a discontinuous distribution such as is described by a Poisson series, a negative binominal (*11*, *21*, *22*), or Neyman's (*15*) contagious distribution, which has been tested for applicability to the field distribution of larval insects by Beall (*3*). Comparisons of the observed and the theoretical Poisson frequency distributions for the first two sampling dates are given in table 1. The χ^2 value in each case denotes a favorable agreement be-

TABLE 1.—*Distribution of 360 field samples classified according to the number of beet leafhoppers on a beet, 1937*

[Fitted Poisson distributions and test of goodness of fit]

Infestation class	May 20			June 4		
	Frequency		χ^2	Frequency		χ^2
	Observed	Calculated		Observed	Calculated	
0	174	161 30	0 9999	263	260 83	0 0180
1	112	129 48	2 3598	81	84 04	1100
2	54	51 97	0793	13	13 54	
3	14	13 01		3	1 45	0500
4	4	2 79		0	12	
5	1	45	4384	0	02	
6	1	06				
7+	0	04				
Total	360	360 00	3 8774	360	360 00	1780
Degrees of freedom			2			1
P_{χ^2}			15			68

tween the observed and the calculated values, and leads to the conclusion that at the time of the spring movement into the beet fields the distribution of the leafhopper is essentially in accordance with the Poisson law. This conclusion is supported by data given by Bowen [5] and by the analyses of an abundance of sampling data (unpublished) obtained in different years by different observers in the sugar beet areas in Idaho, western Colorado, Utah, and California.

The data in table 1 also agree closely with the negative binomial and the contagious distributions. Theoretical values for these distributions, however, are not included in table 1 for the reason that any sample distribution that fits the Poisson must also fit the negative binomial and the contagious. In practice the fit to the latter two usually will be closer than to the Poisson, because for these distribu-

[5] BOWEN, M. F. A METHOD OF ESTIMATING BEET LEAFHOPPER POPULATIONS FROM THE PROPORTION OF UNINFESTED PLANTS. U. S. Bureau Ent. and Plant Quar. Cir. ET-225, 6 pp., illus. 1945. [Processed.]

tions the observed data are forced to agree with theory in the total, the mean, and the variance, whereas for the Poisson, agreement is forced in only the total and the mean.

The variation among the plot totals (fig. 1) may be examined for general conformity to that of a Poisson distribution by the formula given by Fisher (*10*) and Rider (*17*),

$$\chi^2=\frac{\Sigma(x-\bar{x})^2}{\bar{x}}$$

χ^2 so calculated for the 36 plots on May 20 is 59.164, which for 35 degrees of freedom, corresponds to a probability near the 1-percent point.[6] This test therefore indicates a real tendency toward excessive variability in the data. A large contribution to χ^2 is supplied by the one plot in which a total of 18 leafhoppers was recorded. If this plot is omitted from the calculations, the variance is not excessive. χ^2 then equals 46.785, which for 34 degrees of freedom corresponds to a probability of 0.14, approximately. The analysis of variance of the ⅛-acre plots (table 4), which denotes a difference between plots barely exceeding the 5-percent level of significance, also suggests a greater variability among the plot means than would be expected if the distribution of the leafhopper were purely random. Bliss (*4*), in an analysis of data obtained by Fleming and Baker (*12*) on the distribution of Japanese beetle larvae, shows a highly significant variation between plots over an apparently uniform section as small as 18×20 feet (0.008 acre). This condition, observed by Bliss, is more conclusive than appears for the migrant beet leafhopper population here considered, notwithstanding that the experimental area studied was very much larger (4.5 acres) in the case of the leafhopper.

The preceding discussion indicates that there is a tendency for the migrant population of May 20 to depart from a Poisson distribution. This tendency is toward an excess of high and low counts similar to that observed much more distinctly in the resident population of August 26–27, which will be discussed later.

It is reasonable to expect that the true distribution of migrant populations of the beet leafhopper will depart from a Poisson series, because of the many factors both physical and biological that may operate to destroy a perfectly independent distribution of the insect. Air currents or a preference by the leafhopper for certain plots within the field might have caused an uneven distribution of the population on May 20. If this were so, it seems logical to assume that the plot differences would persist during the short period from May 20 to June 4, and that a positive correlation would exist between the plot counts made on these two dates. A cursory examination of the plot totals in figure 1 indicates that no such relationship exists. Actually the correlation coefficient is negative; for the 36 pairs of counts $r=-0.16$, a nonsignificant value, indicating the absence of any relationship between the plot totals of the first two sampling dates. This absence of a significant correlation suggests that the comparatively large number of leafhoppers taken in some of the plots on May 20 might have resulted from chance variations of sampling rather than from a real preference by the insect for certain parts of the field, or from

[6] For degrees of freedom exceeding 30 the expression $\sqrt{2\chi^2}-\sqrt{2n-1}$ is assumed to be a normal deviate with unit standard error.

the effects of physical factors, such as air currents, that may have deposited more leafhoppers in some locations than in others.

The sample distribution of June 4 shows a closer agreement with the Poisson law than does that of May 20, but from the data available it cannot be determined whether this agreement is in fact better. The principal evidence derived from a study of both distributions indicates that the agreement with the Poisson law is essentially satisfactory.

The natural mortality of migrant beet leafhoppers is high. For this reason a decrease in migrant populations always occurs after the spring movement into the fields has ceased, or when this movement is not sufficient to offset the natural mortality in the population already present. Thus, in the field here considered, the leafhopper population decreased from a mean of 0.803 per beet on May 20 to 0.322 per beet on June 4. The fact that on both dates the distribution was essentially in accordance with the Poisson law denotes that the factors affecting mortality of the beet leafhopper acted uniformly in the different plots.

LATER SEASON RESIDENT POPULATION

An attempt to fit the data of August 26–27 to a Poisson series was not successful. This is illustrated by comparing the observed with the calculated Poisson law values given in table 2, and by the corresponding graph in figure 2. In the same table and figure the later season data are also fitted to the negative binomial and the contagious distributions.

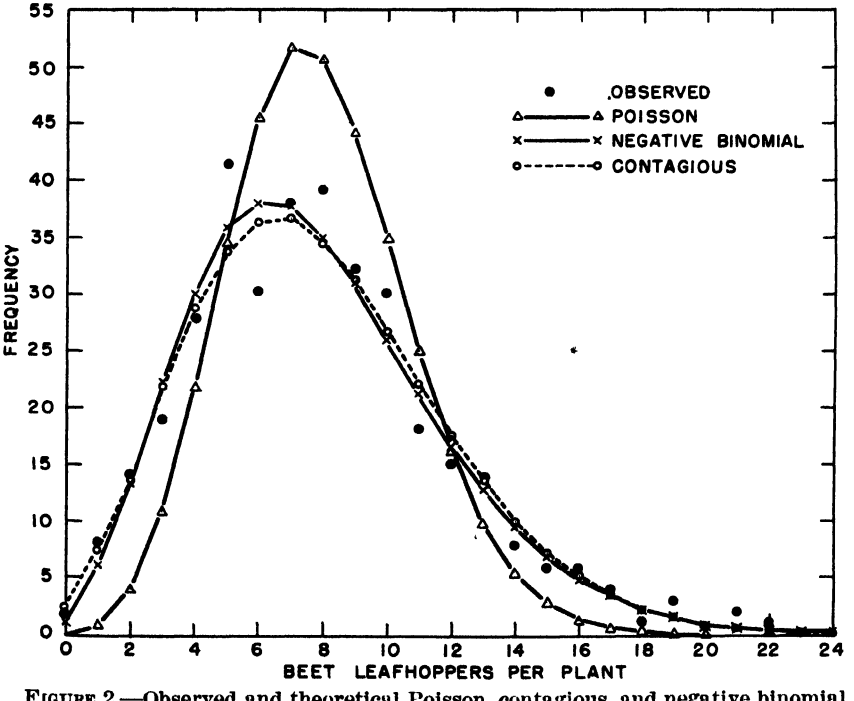

FIGURE 2.—Observed and theoretical Poisson, contagious, and negative binomial frequency distributions of the number of beet leafhoppers per beet in the field samples of August 26–27, 1937. Data from table 2.

TABLE 2.—*Distribution of 360 field samples classified according to the number of beet leafhoppers on a beet, August 26-27, 1937*

[Fitted Poisson, contagious, and negative binomial distributions, and test of goodness of fit]

Infestation class	Frequency				χ^2		
	Observed	Calculated					
		Poisson	Contagious	Binomial	Poisson	Contagious	Binomial
0	2	0.13	2.68	1.62	64.4432	0.0019	0.5765
1	8	1.06	7.46	6.25			
2	14	4.19	14.26	13.66		.0047	.0085
3	19	11.02	21.78	22.24	5.7786	.3548	.4720
4	28	21.75	28.72	29.98	1.7960	.0180	.1308
5	41	34.33	33.78	35.41	1.2959	1.5432	.8825
6	30	45.15	36.46	37.86	5.0836	1.1446	1.6318
7	38	50.90	36.63	37.46	3.2694	.0512	.0078
8	39	50.21	34.65	34.82	2.5028	.5461	.5018
9	32	44.03	31.13	30.75	3.2869	.0243	.0508
10	30	34.75	26.72	26.00	.6493	.4026	.6154
11	18	24.93	22.04	21.20	1.9264	.7405	.4830
12	15	16.39	17.53	16.75	.1179	.3651	.1828
13	14	9.95	13.50	12.87	1.6485	.0185	.0992
14	8	5.61	10.08	9.65	1.0182	.4292	.2821
15	6	2.95	7.33	7.08		.2413	.1647
16	6	1.46	5.20	5.10		.1231	.1588
17	4	.68	3.60	3.61			
18	1	.30	2.44	2.52			
19	3	.12	1.62	1.73			
20	1	.05	1.06	1.17	60.4571	.3784	.0434
21	2	.02	.68	.79			
22	1	.01	.43	.52			
23	0	.00	.27	.34			
24+	0	.01	.22	.62			
Total	360	360.00	360.00	360.00	153.2738	6.3875	6.2919
Degrees of freedom					12	14	14
P_{χ^2}					<.001	.954	.957

The data show a highly significant departure from the Poisson distribution, while the agreement with the negative binomial and the contagious distribution is satisfactory. In fact the χ^2 criterion in the case of these two distributions indicates an even closer agreement between the observed and the calculated values than usually appears for random samples from a homogeneous population. χ^2 values as small as those obtained for the negative binomial and the contagious would appear usually only about once in 23 trials and once in 22 trials respectively. However, such small values might credibly result from chance, and the fit to these distributions is therefore regarded as being not unreasonably close.

It is worthy of note that for this particular sample the values calculated for the negative binomial and the contagious distributions are remarkably similar. Some of Beall's (*3*) data likewise have been found to show satisfactory agreements with both the contagious and the negative binomial.

The contagious and the negative binomial distributions may be regarded as generalizations of the Poisson law for cases in which the variability is excessive as compared to the expectations of a Poisson series. Both of these distributions are characterized by an excess of high and low counts, and both approach the Poisson as the mean and the variance approach equality. The contagious distribution, however, is more flexible than the negative binomial and may sometimes be bimodal, whereas the negative binomial is never bimodal. The negative

binomial is much easier to calculate than the contagious especially when the number of infestation classes is large.

In fitting the observed distributions to a negative binomial $(q-p)^{-n}$, the values of q, p, and n were derived by calculation from the empirical data following the method given by Fry (*13*), i. e., $p=1-V/\bar{x}$, $q=1-p$, and $n=\bar{x}/p$; where V is the variance of the distribution and $\bar{x}$ is the mean infestation per plant. Obviously, the binomial will be negative or positive respectively as the variance is greater or less than the mean. In some samples of migrant beet leafhopper populations, positive binomials ($V<\bar{x}$) have appeared, but in such samples p did not differ significantly from zero (*22*) and the data, therefore, were considered to be in essential agreement with the Poisson law.

In practice, departures from the Poisson will usually be such as to make V greater than $\bar{x}$, although under certain conditions, such as very dense insect populations where there is overcrowding and competition for the available space, a distribution more uniform than the Poisson might result. "Student" (*21*) has studied the effect of departures from the conditions that lead to Poisson's law, and has indicated the conditions in which data may be expected to conform more closely to a negative binomial than to a Poisson series.

The contagious distribution[7] was fitted by using the recurrent formula given by Neyman (*15*) and Beall (*3*),

$$P_{(x=n+1)}=\frac{m_1 m_2 e^{-m_2}}{n+1}\sum_{t=o}^{n}\frac{m_2{}^t}{t!}P_{(x=n-t)}$$

where the initial value, $P_{(x=0)}=e^{-m_1(1-e^{-m_2})}$

The parameters m_1 and m_2 of the above formulae are estimated from the mean and the variance as $\bar{x}^2/(V-\bar{x})$ *and* $(V-\bar{x})/\bar{x}$, respectively.

Later season populations of the beet leafhopper do not agree with Poisson expectations, probably because field conditions at this time do not favor an independent random distribution. Individual plants or certain locations within the field may be more attractive to, or favor a more rapid development of, the insect than others, which condition would upset an independent random distribution and cause the excesses in the small and the large frequency classes (see table 2 and fig. 2) that usually have been observed in distributions that departed from a Poisson series. Part of the contagiousness observed in the resident beet leafhopper population may be attributed to a random dispersion of adults which developed from groups of eggs deposited by the spring migrants. It is doubtful, however, whether this is the primary cause of contagiousness in the field distribution of an insect as active as the beet leafhopper.

The variation in the leafhopper population on August 26–27 cannot be explained by differences in the initial infestation, which, as the analyses of the migrant populations have shown, was essentially uniform. Correlation analyses support this view. The correlation coefficient between the plot totals of May 20 and those of August 26–27

[7] Contagious distribution of type A depending on two parameters. See Neyman 15, pp. 45–48).

was +0.0258, and between the plot totals of June 4 and those of August 26–27 it was +0.0260. Both correlation coefficients are nonsignificant, an indication of the absence of relationship between the initial infestations and the populations that subsequently developed in the different plots within the field.

Data obtained from a field of sugar beets at the Asarco farm, Magna, Utah, in the summer of 1932 illustrate this point. Samples taken in this field on June 20 and 28 showed satisfactory agreement with the Poisson law, and denoted that early in the season the distribution of the leafhopper was quite uniform. Later, however, distinct populations developed in sections of the field characterized by beets of different growth types. This was shown by sampling data taken at weekly intervals from August 6 to 25, inclusive. These data, summarized in table 3, consistently show a significant inverse relationship between the

TABLE 3.—*Beet leafhopper populations on beets of different growth type in field at the Asarco farm, Magna, Utah, 1932*

Date	Leafhopper population per beet [1]			Difference between means		
	Large beets (M_1)	Medium large beets (M_2)	Small wilted beets (M_3)	M_3-M_1	M_3-M_2	M_2-M_1
Aug. 6	1.44±0.21	3.60±0.44	8.48±0.88	7.04±0.90	4.88±0.98	2.16±0.49
11	1.92±.26	4.20±.44	15.44±1.53	13.52±1.55	11.24±1.59	2.28±.51
18	2.52±.33	6.52±.70	23.32±2.13	20.80±2.16	16.80±2.24	4.00±.77
25	3.28±.33	[2]	31.32±2.75	28.04±2.77	-----	-----

[1] Each mean based on counts from 25 beets.
[2] Rain prevented sampling.

density of the leafhopper population and the size of the beets. It is not definitely known just what environmental factors operated to produce the disparate populations on the different-sized beets, but it seems logical to believe that the higher temperatures associated with the small wilted beets favored the production of large leafhopper populations.

In this connection it might be noted that soil heterogeneity may greatly influence the distribution of insect populations indirectly through its action on the plants upon which the insect lives.

VARIATION BETWEEN PLOTS AND WITHIN PLOTS

EARLY SEASON MIGRANT POPULATION

The population data for the 36 plots may be combined to form plots of various sizes and shapes. Twelve size-shape combinations were studied; these are illustrated in figure 3. The variances within and between plots for the data collected on May 20 were computed for each arrangement and are given in table 4.

These analyses were made to determine the effect of plot size on the within-plot variation, which is a measure of the sampling error, and to test whether population heterogeneity was a significant factor affecting leafhopper numbers in the different plots. There is a tendency for the within-plot variance to increase as the subplots become larger, but the effect is small, and the chief evidence is that altering the size or shape of the plot did not materially influence the sampling error.

TABLE 4.—*Analysis of variance of different-sized subplots for field samples of May 20, 1937*

Shape and size of plots		Variance [1]		
Length×width	Area	Between plots	Within plots	*F*
Feet	*Acres*			
91×60	1/8	1 357 (35)	0. 924 (324)	1 47
182×60	1/4	1 191 (17)	955 (342)	1 25
91×120	1/4	1 267 (17)	952 (342)	1 33
273×60	3/8	1 027 (11)	965 (348)	1. 06
91×180	3/8	1 548 (11)	.948 (348)	1 63
(2)	3/8	1 445 (11)	951 (348)	1. 52
182×120	1/2	1 015 (8)	965 (351)	1 05
273×120	3/4	263 (5)	976 (354)	3 71
182×180	3/4	1 203 (5)	963 (354)	1 25
273×180	1 1/8	1 218 (3)	964 (356)	1. 26
546×180	2 1/4	025 (1)	969 (358)	38 76
273×360	2 1/4	225 (1)	969 (358)	4 31

[1] Numbers in parentheses denote degrees of freedom.

[2] 182×60+91×120

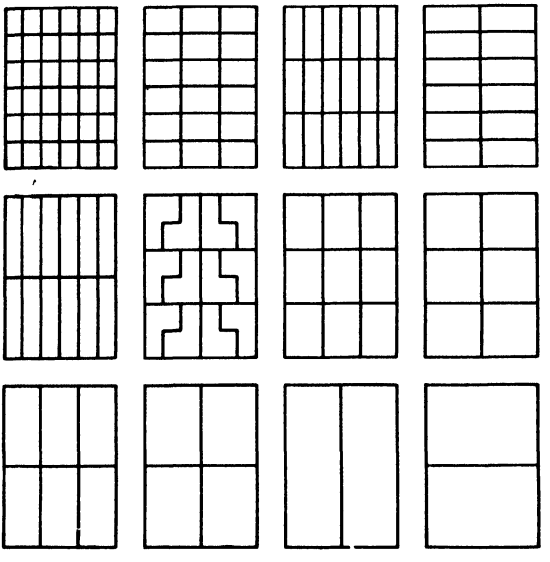

FIGURE 3.—Some of the various sizes and shapes of plots obtained by different combinations of the original 36 plots.

The significance of the difference between plots was determined by the *F* test (*19*). The between-plot variance is barely significant for the 1/8-acre plots, but in no other instance does the observed value of *F* exceed that required at the 5-percent level of significance. The variation between plots was not significantly greater than that within plots, an indication that for the migrant population of May 20 population heterogeneity was not a significant factor affecting the infestation in the different plots. This shows a remarkable degree of uniformity in the leafhopper infestation at this particular time, and corroborates the findings of table 1 which indicate a Poisson distribution.

The data of June 4 show essentially the same condition as those of May 20, and therefore are not considered in detail.

LATER SEASON RESIDENT POPULATION

The population of August 26–27, which resulted largely from the reproductive activity of leafhoppers that migrated into the field prior to June 1, contrasts with the population of May 20. The analyses of variance between plots and within plots for the different arrangements on August 26–27 appear in table 5. The observed value of F for each

TABLE 5.—*Analysis of variance of different-sized subplots for field samples of August 26–27, 1937*

Shape and size of plots		Variance[1]		F
Length×width (feet)	Area	Between plots	Within plots	
	Acres			
91×60	1/8	55.17(35)	11.96 (324)	4.61
182×60	1/4	100 32(17)	11 99 (342)	8.37
91×120	1/4	75 86(17)	13 20 (342)	5 75
273×60	3/8	113.82(11)	13 08 (348)	8 70
91×180	3/8	72. 27(11)	14 40 (348)	5 02
(2)	3/8	108 93(11)	13 24 (348)	8 23
182×120	1/2	148 12 (8)	13 32 (351)	11 12
273×120	3/4	180 69 (5)	13. 84 (354)	13 06
182×180	3/4	129. 32 (5)	14 57 (354)	8 88
273×180	1 1/8	179. 94 (3)	14 79 (356)	12. 17
546×180	2 1/4	97. 14 (1)	15 94 (358)	6 09
273×360	2 1/4	442 22 (1)	14. 98 (358)	29 52

[1] Numbers in parentheses denote degrees of freedom.
[2] Irregular, 182×60+91×60.

comparison except one (546 × 180) exceeds the value required at the 1-percent level of significance, which indicates a highly significant difference between plots, or a marked degree of heterogeneity in the field infestation at this time. There was an appreciable reduction in the sampling error with decreased plot size. Apparently the distribution of the leafhopper at this time was such that for plots of the same size the sampling error is smaller in the long narrow plots than in the short wide ones.

EFFICIENCY OF LOCAL CONTROL IN REDUCING EXPERIMENTAL ERROR

In field-plot experiments involving different treatments the estimate of the error in the experiment is derived from the variation between plots. To illustrate, the total sum of squares between plots in a Latin-square arrangement may be apportioned to the known and controlled causes of variation, i. e., rows, columns, and treatments, the residual sum of squares being due to unknown causes, or experimental error. The variance due to any cause is then determined by dividing the sum of squares by the corresponding number of degrees of freedom.

Table 6 gives a summary of the between-plot analyses of variance of the different-sized plots for the sampling data of the migrant leafhopper population of May 20. Table 7 gives a similar summary for the sampling data of the later season population of August 26–27.

TABLE 6.—*Summary of between-plot analyses of variance for plots of different sizes and shapes and the efficiency of local control in reducing experimental error for data of May 20, 1937*

Treatments × replications	Shape and size of plots		Variance [1]						Error change due to local control.
	Length × width	Area	Total	Rows	Columns	Treatment [2]	Error	Treatment + error	
	Feet	*Acres*							*Percent*
6×6	91×60	1/8	1 357 (35)	1.869 (5)	0 203 (5)	1 223 (5)	1 551 (20)	1 485 (25)	+9.43
6×3	182×60	1/4	1 191 (17)	2 636 (2)		1 123 (5)	.936 (10)	.998 (15)	−16 20
3×6	182×60	1/4	1 191 (17)		.203 (5)	144 (2)	1.894 (10)	1 603 (12)	+34.59
6×3	91×120	1/4	1.267 (17)		136 (2)	.729 (5)	1.763 (10)	1.418 (15)	+11 92
3×6	91×120	1/4	1.267 (17)	1 869 (5)		136 (2)	1 193 (10)	1 017 (12)	−19.73
6×2	273×60	3/8	1 027 (11)	225 (1)		1 656 (5)	.558 (5)	1 107 (10)	+7 79
2×6	273×60	3/8	1 027 (11)		203 (5)	803 (1)	1 896 (5)	1 714 (6)	+66 89
6×2	91×180	3/8	1 548 (11)		.025 (1)	643 (5)	2 758 (5)	1 700 (10)	+9 82
2×6	91×180	3/8	1 548 (11)	1 869 (5)		1 469 (1)	1 243 (5)	1 280 (6)	−17.31
4×3	[3]	3/8	1.445 (11)	2 636 (2)		506 (3)	1 518 (6)	1 180 (9)	−18.34
3×4	[3]	3/8	1 445 (11)		632 (3)	.386 (2)	2 205 (6)	1 750 (8)	+21 11
3×3	182×120	1/2	1 015 (8)	2 636 (2)	136 (2)	.620 (2)	670 (2)	644 (4)	−36.55
3×2	182×180	3/4	1 203 (5)		025 (1)	720 (2)	2 275 (2)	1 497 (4)	+24 44
2×3	182×180	3/4	1 203 (5)	2 636 (2)		625 (1)	058 (2)	247 (3)	−79.47
3×2	273×120	3/4	263 (5)	225 (1)		544 (2)	< 001 (2)	272 (4)	+3 42
2×3	273×120	3/4	263 (5)		136 (2)	336 (1)	353 (2)	347 (3)	+31 94
2×2	273×180	1 1/8	1 218 (3)	225 (1)		3 403 (1)	.025 (1)	1 714 (2)	+40.72
2×2	273×180	1 1/8	1 218 (3)		025 (1)	225 (1)	3 403 (1)	1 814 (2)	+48.93

[1] Numbers in parentheses denote degrees of freedom
[2] Hypothetical.
[3] Irregular, 182×60+91×60

TABLE 7.—*Summary of between-plot analysis of variance for plots of different sizes and shapes and the efficiency of local control in reducing experimental error for data of August 26–27, 1937*

Treatments×replications	Shape and size of plots		Variance [1]						Error change due to local control
	Length×width	Area	Total	Rows	Columns	Treatment [2]	Error	Treatment + error	
	Feet	*Acres*							*Percent*
6×6	91×60	1/8	55.17 (35)	127.06 (5)	145.55 (5)	18.26 (5)	23.83 (20)	22.71 (25)	−58.84
6×3	182×60	1/4	100.32 (17)	253.81 (2)		96.82 (5)	71.37 (10)	79.85 (15)	−20.40
3×6	182×60	1/4	100.32 (17)		145.55 (5)	118.30 (2)	74.11 (10)	81.47 (12)	−18.79
6×3	91×120	1/4	75.86 (17)		216.76 (2)	31.56 (5)	69.84 (10)	57.08 (15)	−24.76
3×6	91×120	1/4	75.86 (17)	127.06 (5)		34.36 (2)	58.57 (10)	54.54 (12)	−28.10
6×2	273×60	3/8	113.82 (11)	442.22 (1)		59.88 (5)	102.09 (5)	80.99 (10)	−28.84
2×6	273×60	3/8	113.82 (11)		145.55 (5)	1.74 (1)	104.52 (5)	87.39 (6)	−23.22
6×2	91×180	3/8	72.27 (11)		97.14 (1)	28.63 (5)	110.94 (5)	69.79 (10)	−3.43
2×6	91×180	3/8	72.27 (11)	127.06 (5)		40.67 (1)	23.81 (5)	26.62 (6)	−63.16
4×3	[3]	3/8	108.93 (11)	253.81 (2)		93.83 (3)	68.20 (6)	76.74 (9)	−29.55
3×4	[3]	3/8	108.93 (11)		188.76 (3)	108.22 (2)	69.26 (6)	79.00 (8)	−27.48
3×3	182×120	1/2	148.12 (8)	253.81 (2)	216.76 (2)	10.86 (2)	95.03 (2)	52.94 (4)	−64.26
3×2	182×180	3/4	129.32 (5)		97.14 (1)	140.81 (2)	133.93 (2)	137.37 (4)	+6.22
2×3	182×180	3/4	129.32 (5)	253.81 (2)		.80 (1)	69.10 (2)	46.33 (3)	−64.17
3×2	273×120	3/4	180.69 (5)	442.22 (1)		72.91 (2)	157.71 (2)	115.31 (4)	−36.18
2×3	273×120	3/4	180.69 (5)		216.76 (2)	23.00 (1)	223.47 (2)	156.65 (3)	−13.30
2×2	273×180	1 1/8	179.94 (3)	442.22 (1)		.47 (1)	97.14 (1)	48.80 (2)	−72.88
2×2	273×180	1 1/8	179.94 (3)		97.14 (1)	.47 (1)	442.22 (1)	221.34 (2)	+23.01

[1] Numbers in parentheses denote degrees of freedom.
[2] Hypothetical.
[3] Irregular, 182×60+91×60.

Inasmuch as the effect of local control is the primary concern of this study, the variances due to rows and columns are of chief importance. No treatments were applied, and the experiment may be regarded as a uniformity trial. Therefore, the treatment effects are purely hpothetical and are presented in these tables only to show the adequacy of the experimental design. It will be noted that seldom is there indicated a significant treatment response. Occasional significant deviations can be expected in a large series of samples even when drawn from homogeneous material. Where only a few large plots are concerned, the treatment effects might be confounded with location differences arising from an unfortunate chance assignment of the treatments to the various plots. Such must be the explanation of the statistically significant treatment effect indicated in line 15 of table 6, and again in the last line of table 7, where there appears to be a significant negative intraclass correlation. Since the treatments were hypothetical the appropriate estimate of experimental error, by which to judge the effects of local control, is obtained by adding the sums of squares for treatment and error and dividing by the combined degrees of freedom. This figure is given in the column headed "Treatment + error."

The effect of local control is measured by the percentage change in the error variance due to the removal of the contributions of rows and/or columns (blocks) from the total variance.

EARLY SEASON MIGRANT POPULATION

Early in the season a field of sugar beets offers an apparently uniform environment. The beets are small and the effect of soil heterogeneity has not yet greatly differentiated them. Previous analyses, given in tables 1 and 4, denoted that on May 20 the leafhopper infestation over the experimental field was essentially uniform, and, therefore, the usefulness of local control when applied to it was highly questionable.

Table 6 shows that restricted designs resulted in an increase in the estimate of experimental error in 12 of the 18 arrangements. In those arrangements where a decrease is indicated, the utility of local control is doubtful, because the variation between blocks usually is not significant and may have resulted from chance. The negative value of restriction, when applied to the migrant beet leafhopper population of May 20, is further emphasized by the fact that the nonsignificant change in the error variance is accompanied by a reduction in the degree of freedom available for the estimate of experimental error. In this connection it should be noted that there was a 66.89 percent increase in the error variance due to the removal of the columns component in the 2×6 randomized blocks design (table 6). The variance of 1.027 is increased by this arrangement to 1.714, and in the process 5 degrees of freedom corresponding to blocks were sacrificed. Clearly the design was very unsuitable.

Although the between-plot analyses for the various arrangements indicate little or no significant effect due to local differences in the leafhopper infestation, it is interesting to compare the row and column variances for plots of the same size and shape. In every arrangement the variation between columns is smaller than that between rows, a

fact suggesting a more uniform distribution of the leafhopper in one direction of the field than in the other. Such a distribution would be likely to develop from an influx of a weak flying species moving from a source along one side of a field, the resulting infestation being heaviest on the side adjacent to the source and gradually diminishing toward the far side.

The beet leafhopper is not a weak flier. Agricultural areas may be infested by dispersals of this insect from breeding sources sometimes hundreds of miles distant (*7*, *18*). It also attains considerable altitude during its flights. Annand and associates (*1*) have shown that during dispersals in southern Idaho large numbers may be found as high as 25 feet at least, and it is not unlikely that much greater altitudes are common. Both of these flight characteristics of the beet leafhopper tend to favor a uniform infestation at the time of dispersal. Over large fields, or very long fields, location differences in the degree of infestation may be considerable. Certainly different fields in the same general area will exhibit highly significant differences in population density. Among other factors, wind currents undoubtedly play an important role in this local distribution, but for areas as small as most sugar-beet fields the effect is apparently small.

From a comparison of the effect of the removal of the row and column variances in the different arrangements it is evident that when a decrease in the error variance occurs it originates from the elimination of the contribution of rows; when an increase occurs it is smaller for the rows than it is for the columns. From this it appears that if a restricted random arrangement were to be used at all in this field at this particular time, the best results would be obtained from randomized blocks laid out across the field in the row direction, not in the column direction.

However, the principal evidence from the analyses of the data of May 20 is that restricted-random arrangements are of little value in field tests made on migrant beet leafhopper populations. This statement probably will apply generally because of the uniformity of the distribution of the leafhopper during the time of the spring flights into the beet fields.

Observations in many fields other than the one considered in this paper have indicated that migrant beet leafhoppers are distributed in a Poisson series. This distribution implies an equal probability of infestation in each of the plots. If the infestations in the plots are alike, it follows that the infestations in different combinations of them will also be alike, and restricted arrangements such as the Latin square or randomized blocks cannot, under such conditions of uniformity, accomplish the purpose for which they are intended.

Migrant leafhopper densities, ranging from a mean of 0.01 to 5 per beet, have been found in essential agreement with the Poisson distribution. At higher densities this distribution law begins to break down owing to an excess of high and low counts, probably because some plants are more attractive to the insect than others. A differential attractiveness among the plants, however, will not necessarily destroy the similarity of the plot infestations, provided the more attractive plants are randomly distributed over the field. This seems to apply to migrant leafhoper populations, so that even at high population densities the practical utility of local control is doubtful.

Further studies, however, are necessary to confirm or refute this belief.

Where migrant populations of the beet leafhopper are concerned it appears that the most satisfactory results will be obtained by a complete randomization of the treatment replications.

LATER SEASON RESIDENT POPULATION

The later season resident population was composed largely of progeny of the spring migrants that moved into the field before June 1. By late August the beets in a field usually manifest obvious differences in growth, and the leafhopper environment is much less uniform than it is early in the season. The environmental differences that develop within the field apparently are reflected in a marked heterogeneity in the leafhopper population.

Population differences associated with types of beet growth have commonly been observed by workers on the beet leafhopper project. Such differences are clearly illustrated by the data obtained at Magna, Utah, in 1932 (table 3). When such obvious differences appear in an experimental field, the investigator might readily take advantage of the associated differences in the leafhopper infestation by orienting the experiment to conform to the various types of plant growth.

Visual orientation of the plots, however, is not always practicable, since marked differences in the leafhopper population may and do appear in the absence of any clearly defined types of plant growth. Such was the case on August 26–27 in the field here considered. Although definite heterogeneity existed in the leafhopper infestation at this time (tables 5 and 7) it would have been impossible, by visual inspection of the field, to associate this heterogeneity with variations in the type of beet growth. Usually it is only by methods of sampling that infestation differences can be recognized with reasonable accuracy.

From table 7 it is evident that local control effected a substantial reduction in the estimate of experimental error, when applied to the resident leafhopper population of August 26–27. In practically all arrangements a decrease in the error variance resulted from removing the variation between rows or columns. In the 6 × 6 Latin square, for example, eliminating the infestation differences between rows and columns reduced the error variance by 58.8 percent; thus, this arrangement would have more than doubled the sensitivity of an experiment as a means of detecting treatment differences.

It is interesting to compare the percentage reduction in the error variances effected by removing rows or columns, when plots of the same shape and size are considered. Removal of the row contribution produced the larger decrease for every arrangement except the 6 × 6 Latin square. In a general way this parallels conditions observed in the data of May 20 except that then the effect of rows, although usually not significant, was larger than that of columns in every arrangement, the 6 × 6 Latin square included.

Although for the data of August 26–27 the rows component was somewhat smaller than that of columns in the 6 × 6 Latin square, in every other arrangement the rows effect was more pronounced. This

is explained by the presence of a definite population gradient across the field in the direction affected by the rows, so that combining the plots in this direction never decreased the differences between rows. There was no definite gradient in the direction affected by the columns, and in combining the plots in this direction the effect sometimes has been to decrease the relative differences. Thus, for the plot 180 feet wide, the error variance was actually increased by eliminating the variation between columns in two instances (182×180 and 273×180), and only an inappreciable reduction occurred in the other (91×180).

DISCUSSION

This study has demonstrated the relationship of insect-population distribution to experimental design. In experiments involving migrant beet leafhopper populations, restricted-random arrangements designed to reduce the experimental error are of little or no value. This results from the uniform distribution of the leafhopper over the experimental area at the time of the spring dispersal into the beet fields. Later season populations exhibit considerable heterogeneity, and the value of local control to increase the accuracy of the results is unmistakable.

As a general rule, in field experiments with any insect species the advantage gained from restricted random arrangements will vary directly with the degree of heterogeneity exhibited by the population sampled. The object of restriction is to include in the location differences as much of the total variation as possible. Obviously this may be accomplished most readily when the population heterogeneity is expressed as a gradient in one or two directions across the experimental field, or lies in definite zones, rather than in patches scattered over the field.

Preliminary samples are usually taken in entomological field tests. Analyses of the preliminary data should indicate the plot arrangement that will most effectively increase the precision of the experiment.

Theoretically, the method of analysis of variance is based on the assumption of a normal distribution. Often this assumption does not hold for field sampling data. Since the distribution of migrant beet leafhoppers is described by the Poisson law, the application of analysis of variance to it is open to question. A similar objection might be made to applying analysis of variance to the later season resident population of August 26–27, which deviates significantly from the normal but conforms favorably with the negative binomial and the contagious distributions. For all practical purposes, however, the variance method applied to tests of significance will probably give reliable results despite a considerable degree of skewness in the data. Experimental studies on nonnormal data by Pearson (*16*) and by Eden and Yates (*9*) support this view. More specifically, Chapman (*5*) has demonstrated a satisfactory agreement between the observed and the theoretical distribution of Z for samples drawn from a Poisson distribution having a mean of 1.0. This approximates the mean (0.803) of the sample distribution of May 20 considered in this report.

In addition to the assumption of a normal distribution, the validity of the generalized standard error derived from a combined analysis of variance is based on the assumption that although the different elements of the population may have different means their variance must be the same. Clearly this assumption may be violated for data of the Poisson type. The variance in a Poisson distribution is not independent, but is equal to the mean. Thus if population heterogeneity is involved, or if different treatments exert a significant effect, the corresponding means, and therefore the variances, will be different, and a combined analysis of the data will be unwarranted.

Considerable caution should be exercised in the analysis and interpretation of such data; otherwise, misleading conclusions may be drawn. Bartlett (*2*) and Cochran (*6*) have dealt at length with this aspect of the problem, and for data of the Poisson type they suggest a square-root transformation to equalize the variances before proceeding with the analysis. For plot counts between 10 and 100 they suggest the simple square-root ($\sqrt{x}$) transformation, and for plot totals the majority of which are under 10 the transformation $\sqrt{x+\frac{1}{2}}$ is recommended.

In the present study, which deals with uniformity data, differential variability was negligible in the populations of May 20 and June 4. A transformation of the data was therefore considered unnecessary. If different treatments had been employed in the experimental field on these dates, then differential variability probably would have been introduced by the treatments, and the $\sqrt{x+\frac{1}{2}}$ transformation would have been appropriate since the plot totals (fig. 1) were below 10 in most cases.

Analyses of variance have been made on the original data and on the transformed data with essentially the same results. Take, for example, the data for the 6×6 Latin square on May 20. When the original figures are used the F values for rows, columns, and treatments respectively are 1.26, 7.32, and 1.27; when the $\sqrt{x+\frac{1}{2}}$ transformation is used the corresponding F values are 1.17, 7.93, and 1.51. Similarly, for the data of the 6×6 Latin square on June 4, when the original data are used the F values for rows, columns, and treatments respectively are 4.49, 1.79, and 1.42; and when the transformation is used the corresponding F values are 4.43, 1.97, and 1.42. Practically the same conclusion regarding levels of significance would be drawn from the analyses of the original data as from the analyses of the transformed data.

The data obtained on August 26–27, besides being non-normal, showed definite heterogeneity and, like those of the first two samplings, are of a type such that the variance and the mean are related. The differences in variability, however, are not so great as to affect appreciably the inferences drawn from the results of analyses performed on the original scale. The analysis of the data for the 6×6 Latin square, when repeated with the simple square-root ($\sqrt{x}$) transformation, gives F values of 5.94, 7.15, and 1.35 for rows, columns, and treatments respectively. These compare with corresponding F values of 5.59, 6.41, and 1.31 when the original figures are used. Both analyses give essentially the same result concerning the significance of the difference between rows, columns, and treatments.

The data considered in this paper further illustrate the practical applicability of the method of analysis of variance to skew (non-normal) distributions (*5*, *9*, *16*), although, by this, it is not intended to imply that a suitable transformation may not often be useful, or even quite necessary, to evaluate correctly the results of an experiment analysed by the variance method.

In this paper field-plot lay-out has been viewed solely from the standpoint of insect-population distribution. Obviously other considerations may be of importance. For example, if it is intended that different insecticides shall be studied, not only as they directly affect the insect but also according to the indirect effect of different degrees of kill on the resulting crop yield, then soil heterogeneity also must be considered. Under this condition restricted-random arrangements would seem advisable, despite uniformity of the insect infestation.

Insect activity and movement may render impracticable the use of designs that otherwise would be very helpful. This difficulty is illustrated by the work of Douglass, Wakeland, and Gillette (*8*) on field experiments for the control of the beet leafhopper in southern Idaho. They showed that a satisfactory kill could be obtained with a pyrethrum oil spray. However, neither the incidence of curly top disease, which is transmitted by the leafhopper, nor the yield of beets per acre in the sprayed plots was significantly different from that in the unsprayed check plots. The beet leafhopper is very active and interplot movements and dispersals into the field after the insecticides were applied completely obscured the effects of the treatment when these were measured by reductions in curly top disease or by increases in the yield of beets. Steiner (*20*) encountered a similar difficulty when he used small plots for field experiments with insecticides for the control of the codling moth. He found that because of intertree and interplot movements of the adult moths, treatment effects might be largely obscured if based only on records from drop and harvest fruit.

SUMMARY

The relation of insect-population distribution to experimental design has been studied by analyses of uniformity data obtained from sampling field populations of the beet leafhopper. (*Eutettix tenellus* (Baker)).

The distribution of the beet leafhopper at the time of the spring dispersal into the sugar-beet fields is in essential agreement with the Poisson law. Later season resident populations apparently conform to the negative binomial and the contagious distributions.

The variation between plots was not significantly greater than the variation within plots for the migrant leafhopper populations, which indicates that population heterogeneity was not a significant factor affecting the infestation in the different plots. The later season resident population showed highly significant differences between the plot infestations.

Restricted random designs gave little or no reduction in error variance when applied to the migrant beet leafhopper populations, because of the uniformity of the distribution of the insect over the

experimental field. These designs, however, effected a significant reduction in the estimate of the error variance when they were applied to the later season resident population.

Precautions to be observed in the application of analysis of variance to data of the Poisson type are indicated.

This paper views field-plot lay-out solely from the standpoint of the distribution of insect populations, but attention is called to the fact that other considerations may be of importance.

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EFFECTS OF CERTAIN SOIL FUNGI AND THEIR BY-PRODUCTS ON OPHIOBOLUS GRAMINIS[1]

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INTRODUCTION

In studies on the take-all foot rot of wheat (*Triticum aestivum* L. (*T. vulgare* Vill.)), caused by *Ophiobolus graminis* Sacc., certain features of its development and disappearance in cultivated fields and of its control by adding organic matter and other amendments to the soil have been difficult to explain. Some workers have suggested that the development of the disease may be affected markedly by other micro-organisms in the soil.

Sanford and Broadfoot (*8*)[2] and Broadfoot (*1*) studied the effect of other organisms on the development of *Ophiobolus graminis* in pure culture and in the soil. Russell (*7*) concluded that the beneficial effect of crop rotation and fallowing on reduction of take-all was due to other soil organisms crowding out *O. graminis* in the absence of its host. Brömmelhues (*2*) reported that several fungi, when grown in artificial media, produced thermostable byproducts that inhibited the growth of *O. graminis* on solid and liquid media. She stated that a preliminary exposure of wheat to certain organisms allowed *O. graminis* to cause more damage than the simultaneous use of those fungi and *O. graminis*. The reason given is that the byproducts of the fungi tested slightly injured the plants and permitted easy infection by *O. graminis*, whereas their byproducts when they were used with *O. graminis* tended to lessen the damage from it. This is cited to explain why take-all is more severe on light than on heavy soil, as the former has less absorptive capacity. Garrett (*5*) stated that the biologic control of the parasite by other micro-organisms best explained some of the phenomena he encountered; Lal (*6*) showed that several soil fungi and bacteria are injurious to *O. graminis*, some by direct attack and others by the deleterious effects of their byproducts; and Winter (*13*) reported the presence in soil extracts of substances inhibitory to the development of *O. graminis*.

Clark (*3*) and Stumbo, Gainey, and Clark (*9*) studied the effect of organic and inorganic soil amendments on the control of take-all. They decided that the control obtained by the amendments they used was due to adequate fertilization of the soil. Their studies of soil flora were only quantitative, however, for no account was taken of the species present or of their byproducts under the circumstances imposed.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 292.

This paper reports attempts to find some of the interrelations between the take-all fungus (*Ophiobolus graminis*) and certain other soil-inhabiting fungi and to ascertain whether variations in the severity of the disease attack by *O. graminis* can be explained on the basis of interference by certain other soil fungi. The paper is not intended as a compendium of the interrelations between a large number of soil fungi and *O. graminis*.

MATERIALS AND METHODS

The fungi studied were isolated from various types of soil used for growing wheat in central Kansas, some being free from *Ophiobolus graminis* and others infested with it. Fungi also were isolated from such infested soils after they had been treated experimentally in various ways in the field and in the greenhouse. Most of the isolations were made from the soil by the poured-plate method, but some were made from root fragments. The media employed included acid and neutral potato-dextrose agar, Lipman and Brown agar, urea agar, nutrient agar, water agar, soil-extract agar, and combinations of water and soil-extract agars.

The fungus isolates were tested for possible antibiosis or probiosis [3] to *Ophiobolus graminis* in artificial media and in the soil. Three series of tests were made. In series 1 *O. graminis* and each of the soil fungi studied were grown together on potato-dextrose agar in 100- by 15-mm. petri dishes. In series 2 the effect of byproducts of the various soil fungi on *O. graminis* was determined. In this series the fungus being tested was grown for 7 to 14 days on a potato-dextrose solution or other liquid media; then the solution was filtered through filter paper and sterilized by autoclaving at 17 pounds' pressure for 20 minutes. Different quantities of the sterile filtrate were then added to fresh, sterile potato-dextrose solution in 125-cc. Erlenmeyer flasks or to sterile, melted potato-dextrose agar in test tubes for use in petri dishes.

Later the flasks of liquid medium and the petri dishes containing the mixtures were inoculated with pieces of agar 2 mm. square cut from portions of an agar plate carrying *Ophiobolus graminis* in an actively growing condition. The flasks and petri dishes were then incubated at 22° to 26° C. The rate of growth of *O. graminis* on the agar plates was determined by measuring the diameters of the circular colonies daily until the plates were covered; it took 7 to 10 days for the colonies to cover the plates if no antibiosis or probiosis was involved. In the liquid media the dry weights of the fungus mats were taken when the experiment was terminated, usually after 14 days. Use of agar plates had the advantage of showing daily increases in growth and of requiring less time, but the use of flasks was less subject to experimental error.

In series 3 the fungi were grown together in variously treated soils which were then planted to wheat. In part of this series the wheat seedlings were grown in the laboratory in 125-cc. Erlenmeyer flasks containing 60 gm. of sterilized soil which had been inoculated with *Ophiobolus graminis* and the fungus to be tested. After *O. graminis*

[3] In the present paper antibiosis is used to mean antagonistic relations of the soil fungi or their byproducts to *Ophiobolus graminis* and probiosis to mean the opposite, that is, favorable relations to *O. graminis*.

had grown through the soil, the fungus to be tested was introduced and the two fungi were permitted to grow together for a time before germinated, surface-sterilized wheat kernels were planted in the flask. At the end of the experiment the extent and severity of root lesions on the seedlings were recorded. In part of series 3 the wheat plants were grown to maturity in the greenhouse in 6-inch clay pots containing soil naturally infested with *O. graminis* and artificially inoculated separately with the different soil fungi to be tested. Data on the extent and severity of the lesions and on the general condition of the wheat plants were taken after they had headed.

FUNGI ISOLATED

Numerous soil and root isolates were cultured and tested for the production of substances that influence the development of *Ophiobolus graminis*. The number of isolates (species or strains) in different genera was as follows: *Penicillium*, 29; *Fusarium*, 20; *Actinomyces*, 17; *Aspergillus*, 16; *Ophiobolus*, 8; *Rhizoctonia*, 7; *Gliocladium*, 6; *Pythium*, 5; *Chaetomium*, 5; *Rhizopus*, 4; *Helminthosporium*, 4; *Alternaria*, 4; *Trichoderma*, 3; *Monilia*, 3; *Trichothecium*, 1; *Acrothecium*, 1; *Spicaria*, 1; and unidentified, 17. These fungi were numbered, and the species of those that tended to be antibiotic or especially probiotic to *O. graminis* were identified whenever possible. These were the principal fungi used in the experiments.

EXPERIMENTS IN ARTIFICIAL CULTURE MEDIA

EFFECT OF OTHER SOIL FUNGI ON OPHIOBOLUS GRAMINIS

In the experiments in which *Ophiobolus graminis* was grown in the same petri dish with another fungus (series 1), potato-dextrose agar was used in duplicate plates. Twenty-seven isolates of common soil fungi were tested separately with *O. graminis*. Each of these and *O. graminis* were placed about 40 mm. apart on opposite sides of an agar plate. The fungus growth, particularly where the hyphae of the two colonies advanced toward each other, was examined several times daily.

A few of the soil fungi studied were actively antagonistic to *Ophiobolus graminis*. These were *Trichoderma lignorum* (Tode) Harz (No. 1), *Aspergillus niger* v. Tiegh. (No. 10), and *Gliocladium fimbriatum* Gilman and Abbott (No. 28). In case of these species the hyphae of *O. graminis* and those of the other fungus advanced on the agar plate until they met. Soon thereafter the hyphae of *O. graminis* died back and disintegrated where the colonies met, and the colony of the other fungus continued to advance. The hyphae of *T. lignorum* actually parasitized those of *O. graminis* by penetrating and killing them. It was not determined how the other two fungi killed the hyphae of *O. graminis*.

Ophiobolus graminis and certain other fungi showed mutual repellence. Both advancing colonies stopped growth at the inside border before the hyphae met, leaving between them a zone where there was no growth. The fungi that reacted in this way were *Aspergillus flavus* Lk. (No. 8), *A. nidulans* (Eidam) Wint. (No. 2), and *Penicillium lilacinum* Thom (No. 13).

The antibiotic or probiotic influence on *Ophiobolus graminis* of the other fungi tested could not be determined exactly by this method because either *O. graminis* grew over them or it was overgrown by them with no apparent injury to either.

EFFECT OF BYPRODUCTS OF OTHER SOIL FUNGI ON OPHIOBOLUS GRAMINIS

Many tests (series 2) were made to determine whether certain soil fungi produced substances toxic or stimulatory to *Ophiobolus graminis*. With few exceptions the medium used was the potato-dextrose solution as described previously. Soil decoctions and Czapek's solution containing various nitrogen compounds were tried also. Measured quantities of the sterile filtrate from the solutions in which individual fungi to be tested had grown (15 percent by volume) were added to a medium in which *O. graminis* was then introduced. Some fungus isolates were tested four or five times and others only once. The resulting growth was recorded as a measure of any possible toxicity. Data were taken both on the increase in diameter of the colonies of *O. graminis* on agar and on the weight of the mycelial mats produced in the liquid medium. The growth of *O. graminis* on the particular medium without the addition of any filtrate was taken as the control.

Examples of both inhibitory and stimulatory effects of various fungi in the test on potato-dextrose-agar plates are shown in figure 1. The average weights of mycelium in liquid medium (potato-dextrose solution) and the measurements of radial growth on agar for 21 of the soil-fungus isolates are shown in table 1. These isolates were selected and identified as to species because they gave a fair representation of the biotic effects encountered.

Thirty-nine, or about one-third, of the isolates included in these experiments produced substances that reduced the growth of *Ophiobolus graminis* more than 20 percent; 20 of these reduced the growth 50 percent or more; and 6 reduced it more than 80 percent. *Aspergillus niger* (No. 10) and *A. terreus* (No. 86) inhibited the growth of *O. graminis* completely.

Four of the six fungi that reduced the development of *Ophiobolus graminis* 80 percent or more were species of *Aspergillus*. According to Thom and Church (*10*) these species fall in the *A. flavus*, *A. niger*, and *A. terreus* groups and have been identified by the authors as strains of *A. flavus* (No. 8), *A. niger* (No. 10), *A. flavipes* (No. 80), and *A. terreus* (No. 86).

On the other hand, eight of the soil-fungus isolates tested in these experiments produced substances that stimulated the growth of *Ophiobolus graminis* 25 percent or more above normal.

The time of production of inhibitory growth substances in artificial media by the soil fungi tested varied considerably. Some produced appreciable quantities in 48 hours and reached their maximum production in 5 days. Others required a week or more to produce any toxic substance and 20 days to attain maximum production. *Rhizopus nigricans* produced inhibitory substances in its early stages of growth and stimulatory ones later. Some isolates of certain species of *Aspergillus*, especially *A. terreus* and *A. niger*, showed wide differences in biotic behavior toward *Ophiobolus graminis*. Such physiologic strains within a species could not always be separated morphologically.

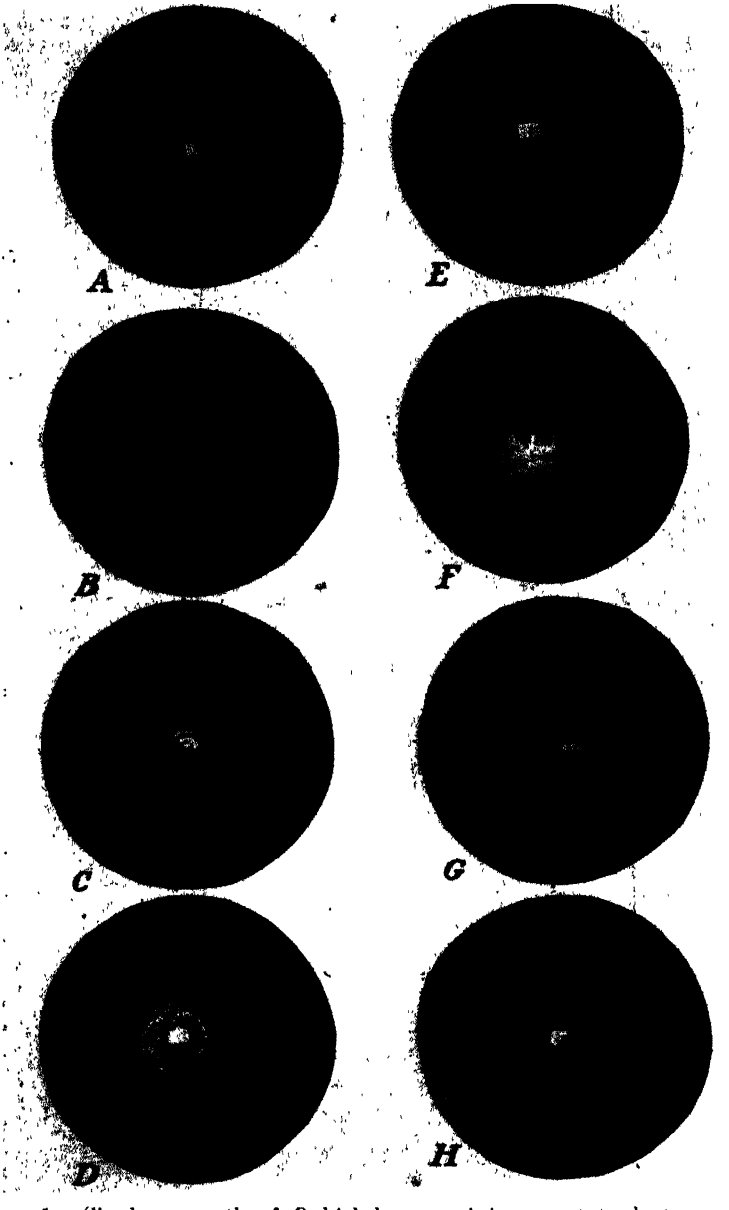

FIGURE 1.—Six-day growth of *Ophiobolus graminis* on potato-dextrose agar to which had been added 15-percent portions of sterile potato-dextrose solutions in which the soil fungi indicated had grown: *A*, *Aspergillus niger* (No. 10), no growth; *B*, *A. flavus* (No. 8), trace of growth; *C*, *Gliocladium fimbriatum* (No. 28), 21 mm. in diameter; *D*, *Trichoderma lignorum* (No. 1), 24 mm. in diameter; *E*, *Penicillium lilacinum* (No. 13), 26 mm. in diameter; *F*, *A. nidulans* (No. 2), 55 mm. in diameter; *G*, *A. terreus* (No. 3), 73 mm. in diameter; *H*, *Alternaria humicola* (No. 17), 85 mm. in diameter. The control colony of *O. graminis* without any added byproduct was 62 mm. in diameter

Two experiments were conducted to test the production of substances affecting the development of *Ophiobolus graminis* by a selected list (table 2) of isolates of soil fungi grown in soil extract. In the first experiment, a preliminary one, soil extracts that had supported the growth of the various soil fungi to be tested were added in small quantities to potato-dextrose agar and potato-dextrose solution; in all cases the growth of *O. graminis* on these media was stimulated markedly. This indicated either that these fungi produced substances in soil extract that stimulated *O. graminis* or that in the soil extract itself there was a growth-promoting substance that obscured the effect of any inhibitory growth substance formed.

TABLE 1.—*Growth of Ophiobolus graminis in potato-dextrose solution or on potato-dextrose agar to which had been added 15-percent portions of sterilized solutions in which various individual soil fungi had grown previously as compared with its growth on the same medium without such addition (control), Manhattan, Kans., 1938*

Fungus grown in solution that was added	Growth of *O. graminis*				
	Weight of fungus mats in solution	Diameter on agar	Compared with growth of control		
			In solution	On agar [1]	Average
	Gm.	*Mm.*	*Pct.*	*Pct.*	*Pct.*
Actinomyces sp. (No. 2A)	0.0574	82	95	117	106
Alternaria humicola Oudemans (No. 17)	.0670	77	111	110	111
Aspergillus flavipes (Bainer and Sartory) Thom and Church (No. 80)	.0089	0	15	0	8
Aspergillus flavus (No. 8)	.0082	25	14	36	25
Aspergillus nidulans (No. 2)	.0553	57	92	81	86
Aspergillus niger (No. 10) [2]	.0000	0	0	0	0
Aspergillus niger (No. 47) [2]	.1082	70	180	100	140
Aspergillus terreus Thom (No. 3) [3]	.0674	77	112	110	111
Aspergillus terreus (No. 86) [3]	.0000	0	0	0	0
Fusarium moniliforme Sheldon (No. 31) [4]	.0968	61	161	87	124
Fusarium moniliforme (No. 109) [4]	.0000	51	0	73	36
Fusarium moniliforme (No. 14) [4]	.0618	69	103	99	101
Gliocladium fimbriatum (No. 28)	.0000	25	0	36	18
Helminthosporium sativum Pam., King, and Bakke (No. 27)	.0667	88	111	126	118
Penicillium lilacinum (No. 13)	.0269	27	45	39	42
Penicillium pinophilum Hedgc. (No. 98)	.0130	25	22	36	29
Rhizopus nigricans Ehr. ex Fr. (No. 4)	.0206	27	34	39	37
Spicaria divaricata (Thom) Gilman and Abbott (No. 116)	.0063	0	10	0	5
Trichoderma lignorum (No. 1)	.0038	37	6	53	30
Trichoderma lignorum (No. 34)	.0066	36	11	51	31
Trichothecium roseum Lk. (No. 29)	.0643	70	107	100	104
Ophiobolus graminis (control)	.0601	70	100	100	100

[1] Percentage computed by comparison of diameters.
[2] These 2 isolates of *Aspergillus niger* could not be separated morphologically.
[3] These 2 isolates of *A. terreus* could not be separated morphologically.
[4] These 3 isolates of *Fusarium moniliforme* could not be separated morphologically.

In the second soil-extract series 5 kg. of garden soil was extracted with boiling distilled water, filtered through cheesecloth, and finally force-filtered through 6 thicknesses of filter paper covered with fine mud. The light-amber liquid obtained was divided into 11 (60-cc.) aliquots in Erlenmeyer flasks and was sterilized 1 hour in the autoclave at 15 pounds' pressure on three successive days. The flasks were then inoculated separately with the following soil fungi: *Alternaria humicola*, *Aspergillus flavus*, *A. niger*, *A. terreus*, *Gliocladium fimbriatum*, *Penicillium lilacinum*, *P. viridicatum* Westling, *Penicillium* sp. (No. 21), *Rhizopus nigricans*, *Trichoderma lignorum*, and *Ophiobo-*

lus graminis. Growth was slow and sparse. After 60 days all flasks were sterilized again by autoclaving, their contents were filtered, and the filtrates were used as 15- and 25-percent (by volume) additions to potato-dextrose agar and potato-dextrose solution in petri dishes and flasks as previously described. *O. graminis* was then grown in each of these. Data on the growth of *O. graminis* in the petri dishes and the flasks were so similar that only those from the flasks are given in table 2.

TABLE 2.—*Growth of Ophiobolus graminis in potato-dextrose solution to which had been added 15- or 25-percent portions of sterilized soil extracts in which various individual soil fungi had grown previously as compared with its growth in the same medium without such addition (control), Manhattan, Kans., 1938*

Fungus grown in solution that was added	Soil extract added	Growth of *O. graminis*	
		Weight of fungus mats	Compared with growth of control
	Percent	*Grams*	*Percent*
Alternaria humicola (No. 17)	15	0.1224	139
	25	.1416	158
Aspergillus flavus (No. 8)	15	.1205	135
	25	.1068	119
Aspergillus niger (No. 10)	15	.1320	147
	25	.1050	117
Aspergillus terreus (No. 3)	15	.1102	123
	25	.1029	115
Gliocladium fimbriatum (No. 28)	15	.1021	114
	25	.0923	103
Penicillium lilacinum (No. 13)	15	.1196	134
	25	.1150	128
Penicillium viridicatum (No. 26)	15	.0999	112
	25	.1430	160
Penicillium sp. (No. 21)	15	.1526	170
	25	.1268	142
Rhizopus nigricans (No. 4)	15	.1047	117
	25	.1492	167
Trichoderma lignorum (No. 34)	15	.1076	120
	25	.1123	125
Ophiobolus graminis	15	.0984	110
	25	.0976	109
Ophiobolus graminis (control)	0	[1] .0895	100

[1] Average of 4 controls.

It is apparent from table 2 that the soil extract which had served as a medium stimulated the growth of *Ophiobolus graminis* in every instance. It is also apparent in the case of *Aspergillus terreus*, *A. flavus*, *A. niger*, *Penicillium* sp. (No. 21), *Gliocladium fimbriatum*, and *Penicillium lilacinum* that the 25-percent addition produced less growth than the 15-percent addition. It might be assumed that the greater amounts of inhibitory material present in the 25-percent addition were responsible for the reduced growth. These particular isolates had been found to be toxic in previous tests in other media, whereas, of the four fungi that exhibited greater growth after the 25-percent addition than after the 15-percent, *Rhizopus nigricans* and *Alternaria humicola* had often produced growth materials stimulatory to *O. graminis* in previous experiments.

Experiments were performed to study the production of byproducts inhibitory to *Ophiobolus graminis* by soil fungi nourished by different sources of nitrogen. Four lots of Czapek's solution were made up, each carrying a different nitrogen compound in concentrations of 2 gm. of the compound per liter. The nitrogen sources used were

ammonium chloride, ammonium nitrate, sodium nitrate, and urea. Potato-dextrose solution was included for comparison. Fifty cubic centimeters of each nutrient solution was placed in 125-cc. Erlenmeyer flasks, plugged with cotton, and sterilized in the autoclave for 1 hour at 15 pounds' pressure. These were then inoculated separately with seven selected soil isolates, namely *Aspergillus flavus*, *A. nidulans*, *A. niger*, *Gliocladium fimbriatum*, *Penicillium lilacinum*, *Rhizopus nigricans*, and *Trichoderma lignorum*. All except *A. nidulans* and *R. nigricans* had been fairly uniform in producing in potato-dextrose solution substances that inhibited *O. graminis*. Each fungus was grown in each of the five solutions for 10 days. All grew except *R. nigricans* with sodium nitrate and *T. lignorum* with ammonium chloride as sources of nitrogen.

After 10 days the solutions were filtered, sterilized, and mixed with potato-dextrose agar or solution so that the fungus extract would constitute 15 percent (by volume) of the mixture. The mixtures were placed in petri dishes and flasks, respectively. These were then inoculated with *Ophiobolus graminis*. As a control *O. graminis* was grown in potato-dextrose solution without the addition of nitrogen compounds and without filtrates containing byproducts of other fungi. The weights of fungus mats from the flasks, which represent the comparative growth, are shown in table 3.

TABLE 3.—*Growth of Ophiobolus graminis in potato-dextrose solution to which had been added 15-percent portions of sterilized solutions in which various individual soil fungi previously had been nourished by different sources of nitrogen as compared with its growth in the same medium without such addition (control), Manhattan, Kans., 1938*

Nitrogen source or medium and fungus grown in solution that was added	Growth of *O. graminis*		Nitrogen source or medium and fungus grown in solution that was added	Growth of *O. graminis*	
	Weight of fungus mats [1]	Compared with growth of control [2]		Weight of fungus mats [1]	Compared with growth of control [2]
Ammonium chloride:	*Grams*	*Percent*	Urea:	*Grams*	*Percent*
Aspergillus niger (No. 10)	0.0000	0	*Gliocladium fimbriatum* (No. 28)	0.0000	0
Aspergillus flavus (No. 8)	.0207	33	*Penicillium lilacinum* (No. 13)	.0523	84
Rhizopus nigricans (No. 4)	.0468	76	*Aspergillus niger* (No. 10)	.0550	89
Penicillium lilacinum (No. 13)	.0501	81	*Aspergillus flavus* (No. 8)	.0594	96
Gliocladium fimbriatum (No. 28)	.0522	84	*Aspergillus nidulans* (No. 2)	.0664	107
Aspergillus nidulans (No. 2)	.0613	99	*Trichoderma lignorum* (No. 1)	.0718	116
Ammonium nitrate:			*Rhizopus nigricans* (No. 4)	.0849	137
Gliocladium fimbriatum (No. 28)	.0000	0	Potato-dextrose solution:		
Aspergillus nidulans (No. 2)	.0524	85	*Aspergillus niger* (No. 10)	.0000	0
Aspergillus niger (No. 10)	.0585	94	*Rhizopus nigricans* (No. 4)	.0072	12
Trichoderma lignorum (No. 1)	.0633	102	*Aspergillus flavus* (No. 8)	.0098	16
Penicillium lilacinum (No. 13)	.0688	111	*Penicillium lilacinum* (No. 13)	.0493	80
Aspergillus flavus (No. 8)	.0942	152	*Gliocladium fimbriatum* (No. 28)	.0568	92
Rhizopus nigricans (No. 4)	.1500	242	*Aspergillus nidulans* (No. 2)	.0603	97
Sodium nitrate:			*Trichoderma lignorum* (No. 1)	.0614	99
Aspergillus niger (No. 10)	.0000	0	Control (potato-dextrose solution alone):		
Aspergillus flavus (No. 8)	.0305	49	*Ophiobolus graminis*	.0620	100
Gliocladium fimbriatum (No. 28)	.0525	85			
Penicillium lilacinum (No. 13)	.0587	95			
Trichoderma lignorum (No. 1)	.0597	96			
Aspergillus nidulans (No. 2)	.0810	131			

[1] Average of 2 replicates.

[2] Fungi listed in each group in descending order of inhibitory action on *Ophiobolus graminis*.

It will be seen in table 3 that the growth of *Ophiobolus graminis* may be variously affected by the byproducts from fungi grown in media with different nitrogen sources. These effects ranged from complete inhibition of *O. graminis* to striking stimulation.

Aspergillus niger grown in Czapek's solution with ammonium chloride or sodium nitrate or in potato-dextrose solution developed byproducts that completely inhibited *Ophiobolus graminis;* only slight inhibition resulted when it was grown with ammonium nitrate or urea. In contrast, *Gliocladium fimbriatum* developed byproducts that effected complete inhibition of *O. graminis* when ammonium nitrate or urea was the nitrogen source but not when the other compounds were used.

Aspergillus flavus when grown in Czapek's solution with ammonium chloride or sodium nitrate or in potato-dextrose solution developed byproducts that inhibited but did not prevent growth of *Ophiobolus graminis*. This is in agreement with the results shown in table 1. In general, the byproducts from *A. flavus* seemed to be of the same nature as those from *A. niger*, but the inhibitory effects were less. It is of special interest to note that the byproducts from *A. flavus* grown with ammonium nitrate were distinctly stimulatory to *O. graminis*, producing 152 percent as much growth as the control.

Rhizopus nigricans developed inhibitory byproducts when grown in potato-dextrose solution or in Czapek's solution with ammonium chloride as the nitrogen source. When ammonium nitrate or urea was used, its byproducts were distinctly stimulatory, causing 242 and 137 percent growth of *Ophiobolus graminis*, respectively.

The byproducts of *Penicillium lilacinum*, *Aspergillus nidulans*, and *Trichoderma lignorum* were only slightly inhibitory, neutral, or slightly stimulatory. The byproducts from these three fungi also had somewhat similar effects in the earlier series, but the inhibition was greater (table 1).

The unused nitrogen remaining in the solutions in which the soil fungi had grown was not responsible for growth stimulation, as Fellows (4) has shown previously that *Ophiobolus graminis* cannot utilize any of the tested sources of nitrogen in a modified Czapek's solution. It is not believed that any of the other unused compounds of Czapek's solution were stimulatory, since *O. graminis* grows better in potato-dextrose solution alone than in modified Czapek's solution even when there is a source of nitrogen in the latter that is favorable for its growth.

The experiment indicates that the production by other soil fungi of substances that are injurious or beneficial to *Ophiobolus graminis* depends on the nature of the soil nutrients as well as on the kinds of fungi present. It indicates that some soil fungi may be distinctly beneficial to *O. graminis* under certain nutritional conditions and decidedly injurious under other conditions. It also suggests that the inhibitory substances produced by one fungus may be different from those produced by another, since they apparently are formed from different materials.

EXPERIMENTS IN SOIL

The interaction of *Ophiobolus graminis* and certain soil fungi was tested both in sterilized, artificially infested soil and in unsterilized, naturally infested soil (series 3). A method was devised for testing

the reaction of *O. graminis* to any other soil fungus by determining the effect of the latter on the pathogenicity of *O. graminis* to wheat seedlings and older wheat plants. In order to avoid introduction of undesirable organic matter, both *O. graminis* and the other soil fungi used in these experiments were cultured in the soil.

EXPERIMENTS IN STERILIZED SOIL

All experiments with sterile soil were in 125-cc. Erlenmeyer flasks containing 60 gm. of soil moistened to approximately 70 percent of its water-holding capacity and steam-sterilized in the flasks. Except in the uninoculated controls the soil was then inoculated with *Ophiobolus graminis* by introducing a 2-mm. cube of agar upon which the fungus was growing. After 14 days' growth of *O. graminis* the soil in some of the flasks was inoculated similarly with the other fungus to be tested. Other flasks were left as inoculated controls, that is, they contained only *O. graminis*. Fourteen days later a surface-sterile wheat seedling was planted in the soil in each flask and allowed to grow 14 days. At that time *O. graminis* had been in the soil 42 days, the other fungus 28 days, and the wheat seedling 14 days. During all this time the inoculated soil and the wheat plant were kept as free as possible from external contamination. The lengths of the three primary roots and of the diseased portions were then measured, and the percentage of root length showing disease lesions was calculated. Twenty-three fungi were tested by this method. Two virulent and one moderately pathogenic strain of *O. graminis* were used. It was found that any fungus that checked the pathogenicity of *O. graminis* checked the moderately pathogenic strain more effectively than it did the highly virulent ones but in the same order. In one experiment seven fungi were used with a moderately pathogenic strain of *O. graminis*. The results are given in table 4.

TABLE 4.—*Effect of certain soil fungi on the growth of wheat roots and on take-all in soil sterilized and then inoculated with Ophiobolus graminis, Manhattan, Kans., 1939*

Fungus added to soil with *Ophiobolus graminis*	Average length of 3 primary roots	Average root length diseased		General appearance and remarks
	Mm.	*Mm.*	*Percent*	
Aspergillus flavus (No. 8)	125	32	26	Fair control.
Aspergillus niger (No. 10)	130	68	52	Good control in 1 flask; none in others.
Fusarium moniliforme (No. 31)	121	22	18	Good control in 2 flask; fair control in 1.
Gliocladium fimbriatum (No. 28)	110	57	52	Some control.
Ophiobolus graminis (control)	117	86	74	Badly diseased.
Pythium arrhenomanes (No. 126)	58	41	70	No control; badly diseased.
Rhizopus nigricans (No. 4)	106	36	34	Doubtful; contaminated with No. 1.
Trichoderma lignorum (No. 1)	114	3	3	Good control.

As shown in table 4, fair to good control of *Ophiobolus graminis* was obtained by artificially inoculating infested soil with *Trichoderma lignorum* (No. 1), *Aspergillus flavus* (No. 8), or *Fusarium moniliforme* (No. 31). Less control was obtained with *A. niger* (No. 10) and *Gliocladium fimbriatum* (No. 28) and none with *Pythium arrhenomanes* Drechs. (No. 126), which is known to be parasitic on wheat. The

flasks containing *Rhizopus nigricans* (No. 4) were accidentally contaminated with *Trichoderma lignorum* (No. 1), obscuring possible effects of the former.

In the uninoculated, sterilized soil the wheat roots and tops were stunted and short but the roots were white and clean. Seemingly there was some toxic effect of steam sterilization of the soil, as the root lengths averaged only 40 mm. as compared with 100 and 150 mm. for those in soils to which fungi had been added. Most of the fungi used, including *Ophiobolus graminis*, dissipated this inhibitory effect rapidly when cultured in the sterilized soil. An exception was *Rhizopus nigricans* (No. 4) in the presence of which the wheat roots were similar to those in uninoculated, sterilized soil. The reason may have been that this fungus inhibited root growth, or it may not have dissipated the injurious effect of steam sterilization, as did the others.

EXPERIMENTS IN NATURALLY INFESTED SOIL

Experiments in which various soil fungi were introduced into soil naturally infested with *Ophiobolus graminis* were rather limited. However, they show that the severity of take-all was reduced by increasing the population of certain species in a soil containing its natural flora.

In one experiment the fungi to be tested were cultured separately under aseptic conditions in moist, sterilized soil in 1-liter Erlenmeyer flasks. Inoculation was accomplished by a 2-mm. block of agar upon which the fungus to be tested was growing. After sufficient growth had occurred, as judged by the penetration through the soil, the culture was mixed with soil naturally infested with *Ophiobolus graminis* at the rate of 1 part by volume of inoculated soil to 3 parts of naturally infested soil. The mixture was placed in 6-inch pots in the greenhouse, and Kanred wheat was planted in it. For the fungi that formed spores, this inoculum was supplemented by adding spores from a petri-dish culture to the top 3 inches of the soil. Planting was done after inoculation. The final examination was made when the wheat plants were nearly mature. Two kinds of controls were used; one consisted of a mixture of 3 parts of naturally infested soil and 1 part sterilized soil and the other of sterilized soil alone.

The effect on the severity of take-all of adding *Aspergillus flavus* (No. 8) to naturally infested and to artificially inoculated soil is shown in figure 2. The seedling roots shown in *A* and *B* grew in soil that had been artificially inoculated with a pure culture of *Ophiobolus graminis*, but the roots in *B* had been protected by *A. flavus* (No. 8), which was added to the soil before the wheat was planted. As shown in table 4, the average root length infected in *A* was 74 percent, whereas that in *B* was only 26 percent.

The control wheat plants, which were almost entirely killed, were grown in soil naturally infested with *Ophiobolus graminis* (fig. 2, *C*). The plants shown in figure 2, *D*, were grown on part of the same lot of naturally infested soil that had been inoculated with *Aspergillus flavus* (No. 8) before the wheat was planted. Although there was some infection, a fair growth of wheat occurred.

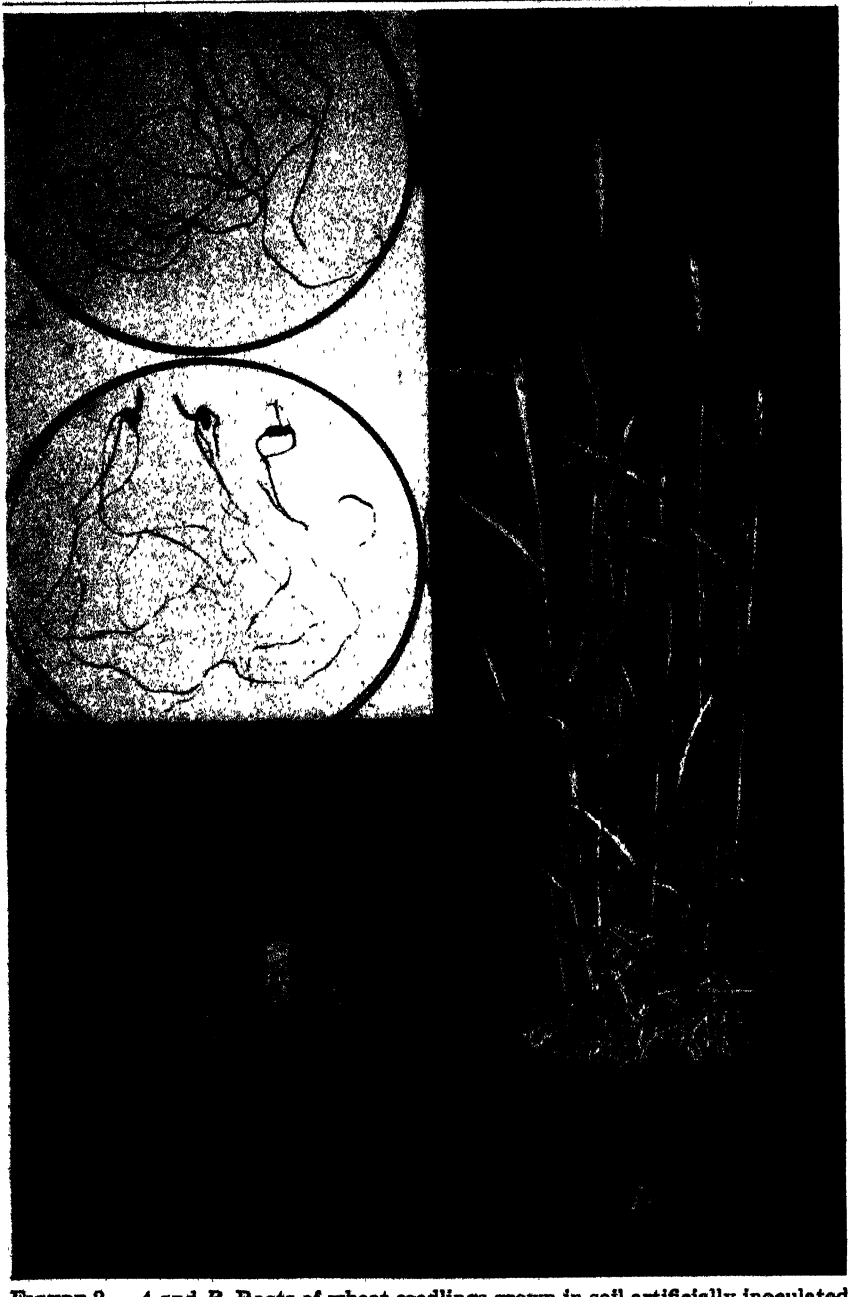

FIGURE 2.—*A* and *B*, Roots of wheat seedlings grown in soil artificially inoculated with *Ophiobolus graminis*: *A*, *O. graminis* alone, 74 percent of root length infected; *B*, with *Aspergillus flavus* (No. 8) added to the soil, 26 percent of length infected. *C*, Wheat plants, almost entirely killed by take-all, grown in soil naturally infested with *O. graminis*. *D*, Wheat plants grown in part of the same soil as *C* with *A. flavus* (No. 8) added to it before the wheat was sown.

DISCUSSION

The data presented herein serve mainly to emphasize the complexities of the problems involved rather than to give any solution. The interrelations of soil fungi, especially when one or more are pathogenic, seem of sufficient importance to merit study, particularly as there are still many unexplained phenomena.

In the present study a number of common soil fungi were found to be antagonistic to *Ophiobolus graminis* in one or more ways and some were stimulatory. Some fungi, *Trichoderma lignorum*, for example, seemed capable of direct parasitism; others under certain conditions produced growth substances distinctly injurious to *O. graminis;* and still others produced byproducts markedly stimulatory to *O. graminis*. Some were capable of either injury or stimulation, depending on the particular set of conditions under which they were grown. Under a given set of conditions, one soil fungus was distinctly antagonistic to *O. graminis* whereas another was beneficial. Under another set of conditions the effects were reversed. In the light of this information, it may be concluded that the applications of certain soil amendments may enable some very common soil fungi to produce byproducts either antibiotic or probiotic to *O. graminis*.

Under the conditions of the experiments at least one fungus (*Rhizopus nigricans*) produced toxic substances during its early growth and markedly stimulatory substances later.

It seems entirely probable that there are several different byproducts of fungus growth capable of inhibiting development of *Ophiobolus graminis*, as such substances are produced from different materials and by different fungi. Furthermore, the production of the byproducts is affected differently by certain physical changes.

The fragmentary evidence obtained in these studies indicates that the metabolism of the soil fungi studied differs widely. For example, *Rhizopus nigricans* was unable to grow in Czapek's solution with sodium nitrate as a source of nitrogen, but it made satisfactory growth when the nitrogen was from other sources. In the same experiment, *Trichoderma lignorum* was unable to grow in Czapek's solution with ammonium chloride as a nitrogen source but it made a normal growth when other compounds were the sources of nitrogen.

It may be noted also that *Gliocladium fimbriatum* and *Rhizopus nigricans* reversed their positions in respect to production of inhibitory byproducts in potato-dextrose solution in the experiments reported in tables 1 and 3. In partial explanation of this seeming discrepancy it may be stated that the 10-day period of growth used in the latter experiment was probably too short for maximum production of inhibitory substances from potato-dextrose media by *G. fimbriatum*. This fungus, which grows rather slowly, required a 20-day growth period for maximum production of toxic substances in potato-dextrose solution, whereas *R. nigricans*, which grows rapidly, required only 5 days to reach maximum production of the inhibitory substances in the same medium. After 15 and 20 days' growth in this medium, however, it was found in several tests to be producing stimulatory byproducts.

The marked stimulation of the growth of *Ophiobolus graminis* caused by byproducts of *Rhizopus nigricans* grown in Czapek's solution with ammonium nitrate as a source of nitrogen is of much

interest. The small quantity of growth solution added could not have furnished any large amount of food. Stimulation must have been in the nature of a hormone, or a "bios." Soil fungi parasitic or inhibitory to other soil fungi have been reported by Weindling (*11*), who also wrote an extensive treatise on association effects of fungi (*12*).

In artificially inoculated soil the parasitism of *Ophiobolus graminis* on wheat roots was definitely lessened by the presence of several other common soil fungi, as shown in table 4. Also in naturally infested field soil the severity of take-all usually was lessened by increasing the population of certain fungi, but the results were less consistent.

SUMMARY

Fungi capable of producing substances that inhibit the growth of *Ophiobolus graminis* in pure culture and of preventing or lessening infection of wheat by *O. graminis* in soil were isolated from soils in central Kansas, both those in which *O. graminis* occurred and those in which it did not occur.

Fungi, the byproducts of which stimulated *Ophiobolus graminis* in pure culture, also were isolated.

Among 143 species and strains of fungi tested, about 25 percent produced in pure culture byproducts that were inhibitory to *Ophiobolus graminis* and about 10 percent produced those that were stimulatory.

The production of inhibitory or stimulatory byproducts by any fungus varied with its stage of growth and the nature of the substrate on which it was cultured. Some fungi produced inhibitory byproducts on one culture medium and stimulatory byproducts on another. On the same substrate inhibitory byproducts were produced by one fungus and stimulatory substances by another.

Several soil fungi decreased the pathogenicity of *Ophiobolus graminis* on wheat in both artificially inoculated and naturally infested soil. The degree of inhibitory action in soil shown by any fungus toward *O. graminis* varied somewhat with the strain of the latter.

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THE EFFECT OF SHORT PHOTOPERIOD ON SORGHUM VARIETIES AND FIRST GENERATION HYBRIDS [1]

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INTRODUCTION

There are numerous varieties of sorghum (*Sorghum vulgare* Pers.) grown in the United States, a score or more of which have been introduced and 40 or 50 of which have been produced through hybridization and selection. In Africa and Asia, where the species is indigenous, there are literally hundreds of varieties. As would be expected, the varietal differences are of many kinds and include contrasting characters involving glume color and texture, awns, of pericarp chloroplast, and other plant colors, types of endosperm starch, integuments, juiciness and sweetness of stem, plant height, rate of tillering, and duration of growth. Many of the differentiating characteristics, including duration of growth, have been shown to be under genetic control. Differences in adaptation other than those influenced by the known genes that determine plant response to photoperiod are also assumed to be genetic. It would be useful to know how sorghum varieties respond to changes in photoperiod since such knowledge would furnish a basis for a genetic classification.

REVIEW OF LITERATURE

The literature concerning the reactions of plants to photoperiod, temperature, and nutrition, and the differential response of varieties to identical treatments has become extensive. It is now generally accepted as a fact that differences in maturity among varieties of many species are brought about by different reactions to environment.

Thompson (*8*) [2] has reviewed the literature that shows the relation between temperature and vegetative and reproductive development in plants. The work in this field shows that the prevailing temperature may determine whether or not a plant will be photoperiodically sensitive. According to Hamner (*3*), most investigators have concluded that if a species contains any strains that can be classed without question in either the long-day or the short-day group, then all other strains of the same species will tend to exhibit responses which would place them in the same group. The varieties or strains of such a species may be arranged in a graded series according to photoperiodic response with the sensitive strains at one end and the more or less day-neutral strains at the other. The inference from this work is that the thermal requirements have not been met whenever a variety or

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[2] Italic numbers in parentheses refer to Literature Cited, p. 300.

strain of a photoperiodically sensitive species does not respond to short (or long) photoperiods.

Just how photoperiodism fits, if it does, into the theory of phasic development is not as yet well understood, as has been pointed out by Hamner (*3*). The concept of phasic development emphasizes the fixed sequence of the phases and the differences between the thermo-scoto-, and photo-phases of plant growth. Contrary to this conception, some evidence indicates that in certain short-day species photoperiodic induction occurs during both the light and dark phases of a cycle. Also, some plants which require a treatment with low temperature have not been reported as behaving like typical short-day plants after low temperature treatment.

Garner and Allard (*2*) showed that sorghum is a short-day species. Borthwick and Parker (*1*) found that a number of soybeans varieties, all of which are sensitive to photoperiod, have different critical photoperiods. Quinby and Karper (*5*) reported that all strains of milo are sensitive to photoperiod and that the strains which have unlike durations of growth apparently differ from one another in having different critical photoperiods.

Sorghum varieties and first generation hybrids between them vary greatly in duration of growth as well as in size and grain production. Karper and Quinby (*4*) have presented data in detail on several varieties and hybrids. The lateness of maturity exhibited by certain hybrids, exclusive of the effects due to heterosis that are discussed by Quinby and Karper (*6*), is considered to be due to the action of complementary genes and it appears that the gene *Ma* is involved wherever extreme lateness occurs.

MATERIALS AND METHODS

When work on the inheritance of genes that affect maturity in sorghum was begun at the Texas station in 1938, it soon became apparent that sorghum varieties differed not only in having different critical photoperiods but also in sensitivity to short-day treatment. To investigate this point further, 12 varieties were grown in a July 3 planting in the field in 1941 at the Chillicothe substation, and part of each variety was subjected to a 10-hour photoperiod and the remainder left without treatment. A similar planting of 14 varieties and 21 first generation hybrids was made on June 30, 1942. During July at this latitude of 34° the sun is above the horizon for slightly over 14 hours. The average minimum (night) temperatures at Chillicothe during July in 1941 and 1942 were, respectively, 71.4° and 70.5° F. The corresponding maximum (day) temperatures were 95.0° and 99.9°.

The plants subjected to 10-hour photoperiods were covered, from the day of planting until after head differentiation of all varieties, from 5 p. m. to 7 a. m. with boxes covered first with rubberized cloth and then with white sheeting. The boxes were constructed in such a way as to allow ventilation. The length of the short-day treatment was 39 days in 1941 and 29 days in 1942. The plantings consisted of short rows about 8 inches apart. Seeds were planted thickly in order that there might be several plants for examination as seedlings and at stages of growth prior to the time of floral initiation and

still leave four plants to grow to maturity. After the time of floral initiation the stand of each variety was reduced to four plants in both treated and untreated plots. In a number of cases there was not sufficient crossed seed to furnish enough plants for examination in the early period of growth, which accounts for the blanks in the tabulated data. When stands were obtained as desired, four plants were present at maturity in a 15-inch row. The stands in the treated and untreated areas, each of which occupied about 45 square feet in 1942, were as nearly identical as possible.

These small areas, which consisted of fertile soil, were watered several times by flooding. Close spacing and ample moisture such as were used in this experiment apparently have no great influence as a part of the environment, since the time of anthesis of the plants under normal day length appears to be within the normal range for the various varieties. Plant size, however, was greatly reduced from that of plants grown in 40-inch rows and 8- to 12-inch spacing. The small plants in the close spacings had the same leaf number as those in wide rows. It appears that close spacing itself does not affect the time that is consumed in laying down an internode but it does affect the size of the growing point which controls the size of the leaves, culm, panicle, etc. Close spacing also retards tiller development.

The fifth and tenth leaves were permanently identified by small tags placed around the stem above the leaf. Each plant was tagged on the day of first anthesis.

The figures that appear in tables 1 and 2 were obtained in the following manner. Plants were examined for floral initiation each day after the twentieth. The first day on which a florally inducted head was found was designated as the day of head differentiation. The figures for days to first antesis and number of leaves were taken from the plant that was selected arbitrarily as being most representative of the four that grew to maturity. To determine whether floral initiation had taken place, plants were split with a pocket knife and the growing points examined under low magnification.

EXPERIMENTAL RESULTS

Data on number of days from planting to head differentiation, first anthesis, and number of leaves of the varieties and hybrids under both normal and 10-hour photoperiods are presented in tables 1 and 2.

It is quite evident that there are differences in sensitivity to 10-hour photoperiods. In 1941 the sensitive varieties differentiated their heads on the twenty-third day and in 1942 on the twenty-first to twenty-third day. This sensitive group of varieties consisted of both milos, Spur and FC 811 feteritas, both hegaris, Freed, California White durra, both kalos, Bonita, Bonar durra, and shallu. Manko was a little less sensitive to 10-hour photoperiods than the group just mentioned but was more sensitive than Blackhull kafir, Sumac, Bishop, and Lemon Yellow. Dwarf broomcorn was the one variety that failed to be affected by 10-hour photoperiods in either year. However, Texas Blackhull kafir was unaffected in 1942, the only year in which it was grown.

The number of days to first anthesis and leaf number are a reflection of the relative time at which head differentiation took place.

TABLE 1.—*Effect of 10-hour photoperiod on time of floral initiation, leaf number, and time of anthesis of sorghum varieties planted July 3, 1941, at Chillicothe, Tex.*

Serial No.	Variety	Number of days from planting to—				Number of leaves on mature plant	
		Head differentiation		First anthesis			
		Short day	Normal day	Short day	Normal day	Short day	Normal day
SA 5043	Sooner milo	23	32	43	49	11	13
TS 25243-276	Texas milo	23	39	47	68	11	18
SPI 34911	Hegari	23	48	47	77	13	18
FC 16207	Kalo	23	39	47	64	11	17
SA 208	California White durra	23	34	51	55	13	14
FC 6601	Spur feterita	23	36	56	65	16	19
SPI 29166	Freed	23	32	46	51	10	12
FC 8991	Manko	25	47	50	77	12	17
FC 8993	Bishop	28	39	61	71	14	17
SPI 35038	Sumac	29	39	60	65	14	16
FC 6607	Blackhull kafir	29	39	59	69	14	16
SPI 30204	Dwarf broomcorn	39	39	68	68	15	15

TABLE 2.—*Effect of 10-hour photoperiod on time of floral initiation leaf number, and time of anthesis of sorghum varieties planted June 30, 1942, at Chillicothe, Tex.*

Serial No.	Variety or hybrid	Number of days from planting to—				Number of leaves on mature plant	
		Head differentiation		First anthesis			
		Short day	Normal day	Short day	Normal day	Short day	Normal day
FC 8975	Sooner milo	22	27	49	49	11	15
TS 25243-276	Texas milo	22	30	46	65	13	17
SA 281	Early hegari	21	30	44	71	11	13
SPI 34911	Hegari	22		46	78	12	15
FC 16214	Early kalo	21	27	46	53	8	12
FC 16207	Kalo	22	29	46	60	11	14
FC 811	Feterita	21	27	45	49	11	15
FC 6601	Spur feterita	23	27	54	62	14	15
SPI 55128	Bonar durra	22	27	51	51	13	15
SA 79	Bohita	22	27	45	51	13	16
Agros. 2650	Shallu			54	75	16	18
TS 24929	Lemon Yellow	27	70	55	110	13	22
FC 8962	Texas Blackhull kafir	29	29	60	60	14	14
SPI 30204	Dwarf broomcorn	29	29	67	64	13	14
SA 1577	Texas Blackhull kafir x Sooner milo	23	25	52	52	12	16
SA 1578	Texas Blackhull kafir x Texas milo	23		52	79	14	22
SA 1594	Bonita x hegari	24		50	80	13	22
SA 1595	Bonita x Early hegari	22		46	79	14	24
SA 1596	Early hegari x Sooner milo	21		41	83	11	20
SA 1598	Hegari x Sooner milo	21		42	80	11	17
SA 1597	Early hegari x Texas milo	22		43	80	13	23
SA 1599	Hegari x Texas milo	22		45	82	12	23
SA 1600	Spur feterita x Sooner milo	21	27	46	52	13	16
SA 1602	Feterita 811 x Sooner milo	22	26	43	45	13	15
SA 1601	Spur feterita x Texas milo	22		48	78	13	22
SA 1605	Early kalo x Sooner milo	22	27	48	51	12	15
SA 1607	Kalo x Sooner milo	22	27	44	49	11	14
SA 1608	Kalo x Texas milo	22	31	44	67	12	16
SA 1593	Bonar durra x Texas milo	22		43	80	12	22
SA 1585	Texas Blackhull kafir x hegari			52	100	13	21
SA 1587	Texas Blackhull kafir x kalo	23	27	46	52	12	14
SA 1588	Texas Blackhull kafir x Maizola	27	27	52	58	14	15
SA 1589	Texas Blackhull kafir x Manko	27	27	53	56	14	15
SA 1591	Texas Blackhull kafir x Bishop	27	28	55	58	14	18
SA 1582	Texas Blackhull kafir x feretita 811	22	25	44	52	13	16

The data, while conclusive, do not appear to be entirely consistent in every particular. Some deviation should be expected since the figures on head differentiation were obtained from plants that were destroyed and the figures on anthesis and leaf number from other plants that grew to maturity. However, the larger number of leaves produced by feterita than by other varieties in the same length of time is typical, as was shown by Sieglinger (7).

Every first generation hybrid differentiated its head along with its sensitive parent. The characteristic of being sensitive to short photoperiod, therefore, acts as a dominant. The only hybrids that approached the nonsensitive parent in lateness of head differentiation were those with two nonsensitive parents.

DISCUSSION

It is assumed that sensitivity to photoperiod indicates that the thermal requirements of the variety have been met. Likewise, it is assumed that the various degrees of insensitivity indicate that the thermal requirements have been met only partially or perhaps not at all. It It was with this idea of sensitivity to photoperiod in mind that the sorghum varieties included in this study were classified into sensitive and nonsensitive varieties. A variety is considered to be sensitive, therefore, if, under 10-hour photoperiods, it differentiates its head, or is florally inducted, in about 21 to 23 days. It is realized that the number of days would vary slightly if elements of the environment other than photoperiod are variable.

Since Texas milo is a later maturing variety than Sooner milo, administering 10-hour photoperiods reduces the time to first anthesis by approximately 20 and 6 days respectively. This difference is not considered to be due to a difference in sensitivity but comes about as a result of the fact that Texas milo and Sooner milo have different critical photoperiods. If this assumption is true, critical photoperiod and sensitivity to photoperiod are distinct manifestations. The differences in maturity and adaptation that characterize the early and late maturing strains of a variety and that result from differences in critical photoperiod are reasonably well understood, but the differences in maturity and adaptation between sorghum varieties that result from differences in sensitivity to photoperiod are not well understood. Differences due to unlike critical photoperiods are such as those between Texas and Sooner milos or between Kalo and Early Kalo; differences due to unlike sensitivity to photoperiod are such as those between Sooner milo and Blackhull kafir. Differences in critical photoperiod differentiate between strains of a single variety, whereas differences in sensitivity differentiate between varieties. The three *Ma* genes whose inheritance has been worked out in milo (5) all influence the critical photoperiod. The inheritance of a gene or genes that influence sensitivity to photoperiod has not been reported, but the differences in inheritance are not simple as in the case of genes that influence critical photoperiod.

It was observed in this and previous studies that once a head is initiated, the size of plant or time to anthesis was fixed even though the plants were not subject to short photoperiod after head initiation. The treated plants produced normal spikelets that bloomed normally and produced a normal set of seeds.

Tillering was greatly reduced by short-photoperiod treatment. As short-photoperiod treatment was discontinued after floral initiation, many plants of tillering varieties tillered even at that late stage. Such tillers were not affected by the fact that the main plant had been florally inducted but grew into large tillers similar to those that develop normally.

Another observation of interest is that in an occasional plant, induction of flowering was delayed a day or two as compared with other plants of the same variety. If short-photoperiod treatment was then discontinued, a plant not florally inducted would continue to lay down leaves and would develop into a plant that, to all appearance, had never been subject to short photoperiods.

SUMMARY

Nineteen sorghum varieties and 21 first generation hybrids were grown from midsummer plantings at Chillicothe, Tex., and subjected to both normal and 10-hour photoperiods.

Sorghum is a short-day species, but the varieties in this study exhibited differences in sensitivity to 10-hour photoperiods. Most varieties, including the milos, kalos, hegaris, feteritas, California White durra, Bonar durra, Bonita, shallu, and Freed were sensitive. Manko was a little less sensitive than these varieties but was more sensitive than Blackhull kafir, Sumac, Bishop, and Lemon Yellow. Dwarf broomcorn in both years and Texas Blackhull kafir in the second year were unresponsive to the short-day treatment.

All first generation hybrids were hastened in maturity when subjected to 10-hour photoperiods if one parent was sensitive. Sensitivity to short photoperiod, therefore, is a dominant characteristic. Hybrids that were relatively insensitive to short photoperiods always had two relatively nonsensitive parents.

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IRREGULARITIES IN A HYBRID BETWEEN TRITICUM DURUM AND T. PERSICUM[1]

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INTRODUCTION

It is rather generally believed that, with the exception of crosses involving *Triticum timopheevi* Zhuk., hybrids between species of wheat with the same number of chromosomes are about as normal as individuals of the parent species. However, several writers, including Aase,[2] Thompson and Robertson,[3] and Hosono,[4] have reported irregularities in certain hybrids. During 1941 and 1942 the writer observed various abnormalities in 67 plants of a hybrid between two species of tetraploid wheat at Columbia, Mo. These irregularities are briefly described herein.

MATERIAL AND METHODS

The species of wheat used in the cross were *Triticum durum* Desf. ($n=14$) variety Kubanka and *T. persicum* Vav. ($n=14$). Most of the observations were made on plants of the parents and the hybrid started simultaneously in a greenhouse in early December 1941 and in early February 1942.

Cytological observations were made on acetocarmine smears made permanent by a tertiary butyl alcohol method described by Sears.[5] Photomicrographs were taken of these permanent preparations and of fresh pollen stained with an aqueous solution of iodine.

MACROSCOPIC AND MICROSCOPIC OBSERVATIONS ON PARENTS AND HYBRID

The parents and F_1 plants developed at about the same rate. At maturity the height, number of culms, and seed production of each

[1] Received for publication May 12, 1947. This work was carried out in cooperation with the Missouri Agricultural Experiment Station. Contribution from the Field Crops Department, Missouri Agricultural Experiment Station, Journal Series Paper No. 1016.

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[5] SEARS, E. R. CHROMOSOME PAIRING AND FERTILITY IN HYBRIDS AND AMPHIDIPLOIDS IN THE TRITICINAE. Mo. Agr. Expt. Sta. Res. Bul. 337, 20 pp., illus. 1941.

plant were recorded. These data, summarized in table 1, give little indication of heterosis in the F_1 plants as measured by the three criteria. The seed production of the F_1 plants was even less than that of the parents. The reduction probably was due to the greater sterility of the hybrid plants.

The relative fertility of the 2 species and the F_1 hybrid is shown in table 2. The hybrid plants had 3 times the percentage of sterility of *Triticum persicum* and more than 10 times that of *T. durum*.

TABLE 1.—*Average height, number of culms, and seed production of plants of Triticum durum, T. persicum, and the F_1 hybrid*

Stock	Plants observed	Height	Culms	Seed produced
	Number	*Centimeters*	*Number*	*Grams*
T. durum	13	127	4.7	6.66
T. persicum	12	107	6.9	6.96
T. durum × *T. persicum*	12	126	5.7	5.53

TABLE 2.—*Fertility of plants of Triticum durum, T. persicum, and the F_1 hybrid*

Stock	Plants observed	Florets			
		With seeds		Without seeds	
	Number	*Number*	*Percent*	*Number*	*Percent*
T. durum	6	610	98.1	12	1.9
T. persicum	8	840	92.8	65	7.2
T. durum × *T. persicum*	6	388	78.5	106	21.5

Observations on mature pollen revealed a similar relation. About 39 percent of the pollen in the hybrid plants was visibly defective as compared with 11 percent in *Triticum persicum* and 5 percent in *T. durum* (fig. 1 and table 3).

TABLE 3.—*Pollen counts on plants of Triticum durum, T. persicum, and the F_1 hybrid*

Stock	Plants observed	Pollen grains filled with starch as indicated—					
		Well filled		Partially filled		Empty	
	Number	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
T. durum	3	1,185	94.6	21	1.7	46	3.7
T. persicum	2	829	89.1	14	1.5	87	9.4
T. durum × *T. persicum*	3	997	60.6	149	9.1	499	30.3

Cytological observations on microsporogenesis revealed irregularities in the F_1 plants (table 4). The most conspicuous abnormality was a quadrivalent which was present in each pollen mother cell (fig. 2). In about one-third of the pollen mother cells the quadrivalent occurred in the form of a chain. From the observations of Thompson and Thompson [6] it appears that the greater sterility in the hybrid (table 2)

[6] THOMPSON, W. P., and THOMPSON, M. G. RECIPROCAL CHROMOSOME TRANSLOCATIONS WITHOUT SEMI-STERILITY. Cytologia (Fujii Jubilaei Vol.) 1937: 336–342, illus. 1937.

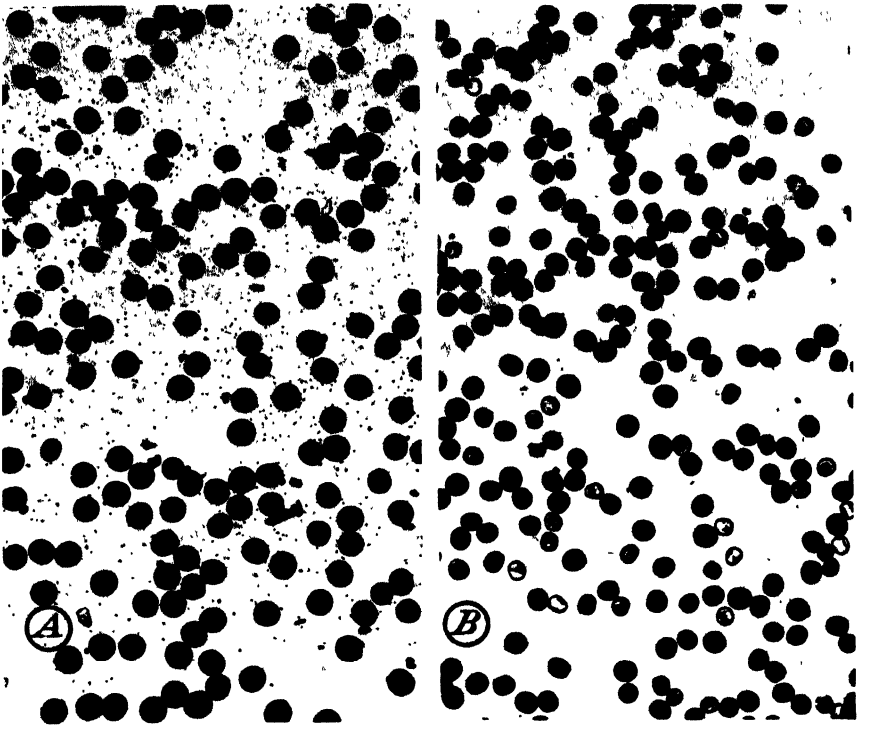

FIGURE 1.—Mature pollen stained with iodine: *A*, *Triticum durum*; *B*, F_1 hybrid (*T. durum* × *T. persicum*). Note that the pollen grains of the hybrid were smaller and that a greater proportion of them were incompletely filled or empty. × 70.

TABLE 4.—*Cytological observations on meiosis in Triticum durum, T. persicum, and the F_1 hybrid*

Stock	Microsporocytes with arrangement of chromosomes as indicated—									Quartets with micronuclei				
	Open bivalents					Univalents		Quadrivalents						
	0	1	2	3	4	0	2	0	1	0	1	2	3	4
	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number
T. durum	39	18	9	0	0	66	0	66	0	205	11	0	0	0
T. persicum	13	22	7	3	1	46	0	46	0	335	49	14	1	0
T. durum × *T. persicum*	20	19	20	9	1	67	2	0	69	551	45	25	7	2

probably cannot be attributed to the quadrivalent. Open bivalents also were more common in the hybrid than in the parents (1.3 per cell as compared with 1.1 per cell in *Triticum persicum* and 0.55 in *T. durum*). Univalents were rare in the hybrid, but none was observed in the parent species. There was no indication of inversions in the

chromosomes of the F_1 plants. Micronuclei in the quartets resulting from the second meiotic division were about equally frequent in *T. persicum* and the F_1 (table 4). In both they were about four times as frequent as in *T. durum*.

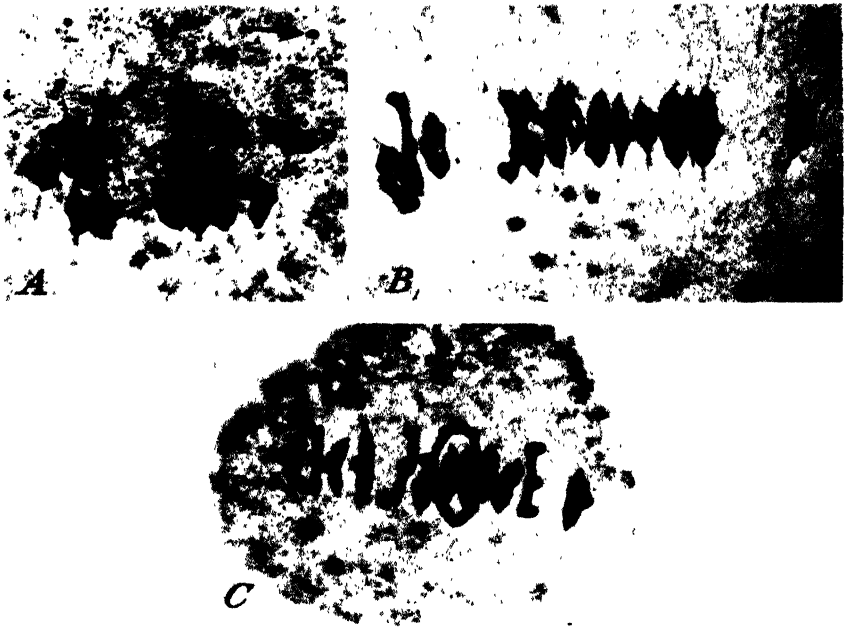

FIGURE 2.—*A*, Pollen mother cell of *Triticum durum* at first meiotic metaphase. Note the 14 closed bivalents and the tiny fragment (arrow) which was also regularly present in this plant. *B*, Pollen mother cell of *T. durum* × *T. persicum* at first meiotic metaphase. Note the chain quadrivalent and the 12 bivalents, of which 1 is open. *C*, Pollen mother cell from the same individual as *B*, showing a ring quadrivalent and 3 open bivalents. × 850.

A fragment similar to that in figure 2, *A*, has been observed by the writer [7] in common wheat.

In addition to the irregularities mentioned, there were other but less definite abnormalities in the F_1 plants. Metaphase plates were not neat and orderly. The chromosomes were not well-defined and regular in outline, and the bivalents and the anaphase chromosomes were frequently stuck together. In general the pollen mother cells of the F_1 plants presented a more disorderly appearance than those of the parent plants, although lagging and loss of chromosomes or formation of bridges at first anaphase did not appear to be any more frequent in the hybrid plants than in the parents.

DISCUSSION

It is evident that the stocks of *Triticum durum* and *T. persicum* used in this study had differentiated in other ways than morphologically. It would be surprising if this were not so. In most cases the accumulation of differences that characterize these and other species

[7] Unpublished observation.

could take place only if aided by geographic or genetic isolation. This differentiation exhibited itself in chromosomal irregularities, partial sterility, and possibly other irregularities in the hybrid plants. Such disorders probably act with linkages in interfering with the transfer of characteristics such as quality, disease resistance, winter hardiness, and yield from one species to another, even though the species have the same number of chromosomes.

In a number of respects *Triticum persicum* was intermediate between *T. durum* and their hybrid. *T. persicum* had more defective pollen, greater sterility, and a higher frequency of meiotic irregularities than *T. durum*. The irregularities in *T. persicum* and the hybrid were probably due to both genetic and physiologic causes.

The apparent absence of heterosis in this species cross is of interest.

SUMMARY

Observations on plants of a hybrid between two tetraploid species of wheat (*Triticum durum* and *T. persicum*) revealed a number of irregularities, although the number of chromosomes in the two species was the same. Meiotic abnormalities included a quadrivalent and other but less readily analyzable peculiarities. Plants of the hybrid exhibited no evidence of heterosis and were partly sterile, possibly because of physiologic as well as genetic factors. It is probable that such irregularities in hybrids between species with the same number of chromosomes are more common than is generally recognized.

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